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The ecological genetics of growth in *Drosophila*

3. GROWTH AND COMPETITIVE ABILITY OF STRAINS SELECTED ON DIFFERENT DIETS

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(*Received* 28 *November* 1959)

1. INTRODUCTION

The preceding paper in this series (Robertson, 1960*b*) dealt with characteristic differences in selection response for large body size when the larvae were supplied with different diets. Individual variation in adult body size represents—to a varying degree—the effects of genetic segregation and recombination on different processes of growth and metabolism when the conditions during larval growth differ. Hence selection for the same 'character' in different conditions leads to qualitatively different changes in the physiology of growth and this was advanced as the essential reason for the differing responses to selection on different diets. If this interpretation is valid it should be possible to provide further direct evidence by comparing the growth of such contrasted strains under different controlled conditions. Such comparisons should also throw light on genetic differences in the ability to maintain a characteristic body size as well as inter-relations between development time, body size and diet.

Accordingly the growth response has been determined for unselected flies and for three large strains created by selection either on the live yeast medium or on aseptic, synthetic diets, which were either deficient in protein or had the concentration of all nutrients reduced. These comparisons have been carried out after six to eight generations of mass selection on the live yeast medium and after six to twelve generations on the two deficient diets. Since the response to selection in the latter case ceased after seven or eight generations, and there is no reason to suppose that the performance of the lines on alternative diets changed thereafter, the comparisons can be taken to represent what happens after about six to seven generations of mass selection under such different conditions.

It has been noted, in the preceding paper, that the response to selection on the deficient diets involved better adaptation to these conditions. This was inferred from the decline in variance of adult size, the shortening of development time and also the increase in egg production—compared with unselected flies—which accompanied effective selection. Hence, these large strains can be regarded, to some extent, as adapted to different diets.

2. MATERIALS AND METHODS

The media upon which larvae have been grown aseptically comprise either synthetic Medium C of Sang (1956) or various modifications of it which include: (*a*) omission of the fructose, (*b*) reduction of the RNA level from 0·36 to 0·09%, (*c*) reduction of the casein concentration from 5% to either 4, 3, or 2%, (*d*) various combinations of these specific deficiencies and also (*e*) general dilution of Medium C to one-third normal concentration, except for the agar gel. Circumstantial evidence suggested that deficiencies of this kind might not be too far removed from the kind of adverse conditions commonly encountered by the population. In addition, the strains have been compared under competitive conditions on the live yeast medium, but the procedure in this case will be described later.

Body size is generally based on the average of eight females drawn from four or five replicated cultures. Since development time is scored on all females hatching from the cultures, there are more degrees of freedom for this measure of growth than for body size. The latter is based on the length of thorax, while development time is calculated from records of the morning and evening hatch of adults over successive days. The average duration of the pupal period is subtracted from the total duration of development to estimate the larval period. All data have been transformed to a log scale. Body size is expressed as three times the natural log of thorax length in 1/100 mm. while the larval period is reckoned in log days. Further details of procedure and general orientation are given in the introductory paper of this series (Robertson, 1960*a*). The population of *Drosophila melanogaster* used in these experiments is known as *Pacific* and had been run for about a year in a population cage before the start of the selection experiments.

3. RESULTS

The data are dealt with in three sections. Firstly, we have comparisons between the unselected population and the strain selected for large size in the low-protein medium; this includes the effect of backcrossing this strain to the unselected population. Secondly, we have comparisons of growth on synthetic diets, which differ in protein concentration, between the unselected population and the three large strains selected on different media. Finally, all the strains are compared under competitive conditions on the live yeast medium.

(i) *Tests on the low-protein strain*

(*a*) *Comparison with the unselected population*

After six generations of selection for large body size on the low (2%) protein diet, i.e. a generation before the response to selection ceased, eggs were collected from the selected strain and from the unselected population, and were set up on the live yeast medium and also on six different synthetic media. These were as follows: Medium C; (*a*) 'complete', (*b*) without fructose, (*c*) with low RNA, (*d*) with 2% casein, (*e*) with 2% casein and no fructose and (*f*) with 2% casein and low

RNA. The deviations from the performance of the unselected flies for the seven treatments are set out in Table 1.

In this and later Tables, which refer to the deviations of various strains from

Table 1. *Performance of the large strain selected on a low-protein diet, compared with unselected flies*

Medium	Deviations from unselected—logarithms	
	Body size	Larval period
Live yeast	0·12	0·01
5% casein	0·16	−0·01
5% casein, no fructose	0·21	0·03
5% casein, 0·09% RNA	0·15	−0·01
2% casein	0·17	−0·05**
2% casein, no fructuse	0·19	−0·01
2% casein, 0·09% RNA	0·21	−0·02

** indicates significance at the 0·01 level of probability.

their performance on the live yeast medium or from the performance of unselected flies on different diets, statistical significance of the differences are noted only for the larval period, since the deviations for body size are almost invariably highly significant; the few exceptions are noted in the text. In addition, the deviations from unselected flies on different diets, listed in columns in the Tables, have been shown to be highly heterogeneous for each set of comparisons. Hence gene-environment interaction is widespread and dramatic in magnitude. In dealing with such effects a statistical index of interaction—although useful as a first indication of a problem to be solved—is biologically meaningless and the origin of such differences has to be sought in terms of characteristic changes in growth.

It is obvious that the selected and unselected strains differ in how much body size is reduced on different diets; the striking heterogeneity of the differences has been confirmed by the usual statistical tests. Difference in body size between selected and unselected is least on the live yeast medium (12%) and greater on all the deficient diets, especially those deficient in fructose or RNA, in which the difference amounts to some 20%. It may be noted that the reduction of RNA from 0·36 to 0·09% in the presence of 5% casein leaves the difference between strains unchanged, while the same reduction of RNA, in the presence of a lower protein level, increases it—a good example of how the limiting effect of a given nutrient in short supply is influenced by the concentration of other constituents of the diet.

These differences in body size arise from differences in growth rate rather than alteration in the duration of the growing period. Thus, the selected strain, which may be some 20% greater than the unselected, either does not differ in average development time or tends to develop a little faster, especially on the 2% protein medium used for selection.

The interpretation of these results is clarified by reference to Fig. 1, in which

body size is plotted against larval period on the log scale. Taking the performance on the live yeast medium as the reference point, Medium C, both with fructose (series 2) and without fructose (series 3) leads to a considerable reduction of body in size the unselected flies, whereas in the large strain there is no reduction below the maximum size. This capacity for maintaining body size constant is encountered when the diet is not too adverse. Development time may be prolonged to a variable degree so that, under such conditions, there is no environmental correlation between these two measures of growth. But when the diet becomes too inadequate body size declines and there is established an inverse relation between size and

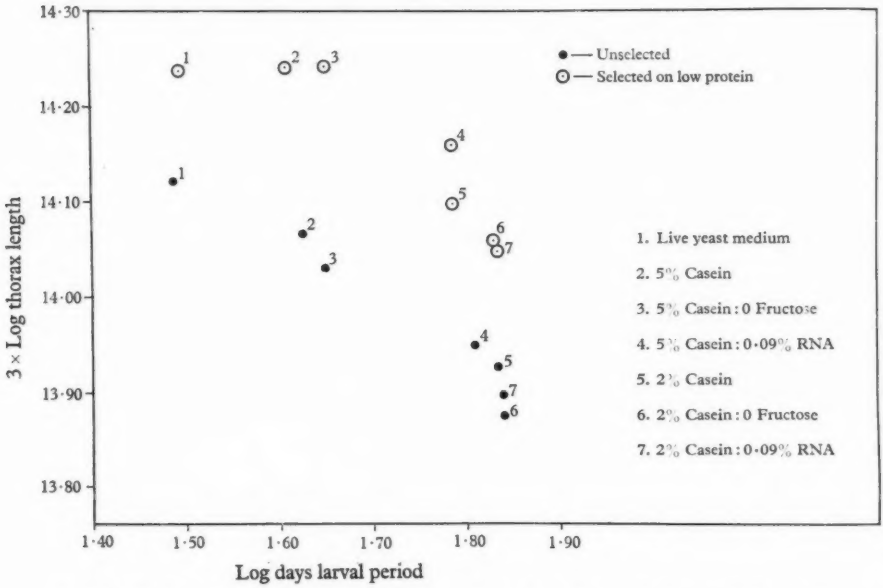

Fig. 1. Body size and development time of unselected population and the strain selected for large size on low protein, when grown on various media.

development time such that a given decline in size is accompanied by a more or less proportional increase in developmental time, as noted earlier (Robertson, 1959 and 1960*a*). In such situations the environmental correlation is very high. It is quite clear that the selected strain has much greater capacity to maintain a constant body size on diets which lead to about 10% reduction in the body size of the unselected flies. But when the diet is sufficiently sub-optimal to reduce body size of the selected individuals there is no evidence that they are better able to resist these more adverse conditions, than the unselected individuals, by relatively greater lengthening of the larval period.

Although sub-optimal diets reveal the inverse relation between decline in size and increase in development time, there is variation about the regression line due apparently to the specific composition of the diet. Thus, for the unselected flies,

treatments numbered 5, 6 and 7 in Fig. 1 lead to appreciable differences in body size but to about the same average development time. It appears that under certain conditions, body size may be reduced without further delay in development. It is likely that such contrasts originate in characteristically different effects of such diets on particular stages of larval growth. This general problem is being studied in further experiments.

(*b*) *Performance of the backcross*

After seven generations of selection on the low-protein medium, selected flies were crossed to the unselected stock and the F_1 tested at the same time as the two parental strains on the live yeast medium and on Medium C: (i) without fructose, (ii) with 2% casein, (iii) with 2% casein and 0·09% RNA and (iv) Medium C diluted to one-third strength.

Table 2. *Selected, unselected and cross on different diets; deviations from body size on live yeast medium*

	Genotype			Difference in response	
Medium	Unselected (U)	Selected (S)	Cross (X)	U − S	U − X
No fructose	−0·04	0·03	0·00	−0·07**	−0·04*
2% casein	−0·24	−0·13	−0·15	−0·12**	−0·09**
2% casein, 0·09% RNA	−0·25	−0·11	−0·22	−0·14**	−0·03
Diluted	−0·23	−0·17	−0·21	−0·06**	−0·02

* and ** indicate significance at the 0·05 and 0·01 level of probability.

The results are shown in Table 2 and Fig. 2. Comparison of Fig. 2 with Fig. 1 shows that development time was considerably longer in the later test. This is almost certainly due to the greater exposure to autoclaving in this experiment. As noted in the introductory paper, differences in performance of the same strain set up on the medium made to the same formula are almost certainly due to variation in degree of heating, which cannot be controlled as precisely as would be desirable, with the equipment available.

This test shows that the body size of the selected strain is actually greater on the fructose-deficient medium than on live yeast (the difference is significant at the 0·05 level), compared with the unselected flies which are reduced on this medium. The cross is apparently intermediate and its body size does not differ on the two diets. Differences between strains in reaction to the deficient media are listed on the right of Table 2. The individual differences between body size on live yeast and other diets are tested against the sum of the four appropriate variances of a mean.

Figure 2 and Table 3, which summarizes the deviations from mid-parent value for both body size and larval period, show that the F_1 is generally intermediate. However, on the dilute medium, which causes about the same reduction of body size as 2% casein, but on which the larval period is considerably shorter, the F_1 is 3% smaller than the mid-parent value—a statistically significant difference.

With respect to larval period, none of the deviations from intermediacy are significant, but all are negative on the aseptic media, suggesting that the cross develops at a slightly faster rate than the parents.

The graph relating body size and development time shows the familiar features

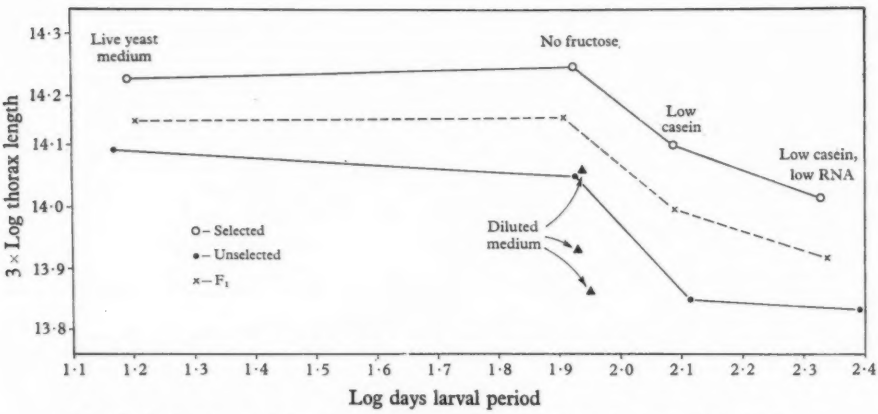

Fig. 2. Body size and development time of the unselected population, the large low-protein strain and the F_1 between them when grown on alternative media.

of lengthened development time and comparatively constant size on the least sub-optimal diet and the inverse relation with more extreme conditions. However, in this test, when the protein level is low, reduction of the RNA level does not further reduce the body size of unselected flies although it lengthens the larval period. In the selected strain, body size is reduced as well while the cross is more or less intermediate in this respect. The difference between the reaction of unselected

Table 3. *Deviation from mid-parent value in backcross*

Medium	Body size	Larval period
Live yeast	−0·02	0·01
No fructose	0·01	−0·01
2% casein	0·02	−0·02
2% casein, 0·09% RNA	−0·01	−0·02
Diluted	−0·03*	−0·01

* indicates significance at the 0·05 level of probability.

individuals to RNA reduction, in the presence of low protein, in this experiment and the one summarized in Fig. 1 is probably due to differences in exposure to autoclaving.

(ii) *Comparisons of large strains on protein-deficient media*

At the end of the various selection experiments, i.e. after 7, 12 and 9 rounds of selection on respectively live yeast, 2% casein and the diluted medium, eggs from

Table 4. *Deviation from unselected of large strains on different protein-deficient diets—body size* (S) *and larval period* (L) *on log scale*

	Medium used during selection of large strains					
	Low protein		Diluted		Live yeast	
Medium of test	S	L	S	L	S	L
Live yeast	0·14	0·02	0·17	0·01	0·14	0·07**
Aseptic						
6% casein	0·23	0·04	0·25	0·06*	0·11	0·13*
5% casein	0·19	0·04*	0·24	0·01	0·12	0·14*
4% casein	0·25	−0·07	0·27	−0·01	0·06	0·14*
3% casein	0·27	−0·09	0·30	−0·10	0·13	0·09*
2% casein	0·29	−0·02	0·30	0·09	0·06	−0·01
Diluted	0·29	0·03	0·32	0·08*	0·02	0·23**

* and ** indicate significance at the 0·05 and 0·01 level of probability.

the unselected stock and from the large strains were set up on the live yeast medium, on the aseptic medium with either 6, 5, 4, 3 or 2% casein, and also on the diluted medium. At the same time eggs from these flies were allowed to hatch into larvae which were set up under competitive conditions on a modified live yeast medium. The response to varying protein concentration will be considered first.

The results are shown in Table 4 and Fig. 3.

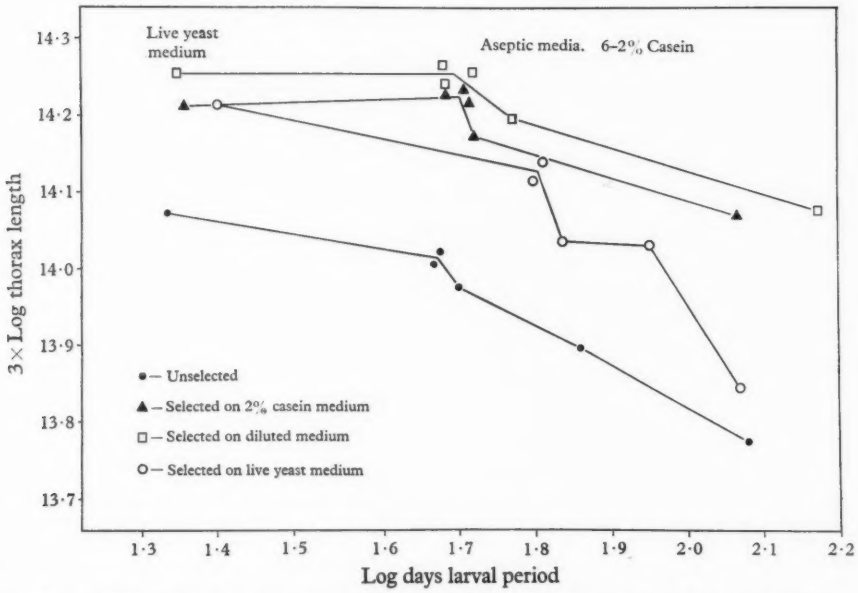

Fig. 3. Body size and development time of the unselected population and three large strains selected on different media, when grown on the live yeast medium and also on synthetic diets with different concentrations of protein.

The main features are as follows:

(i) On the live yeast medium all three strains are about the same size—some 15% greater than unselected flies. This is a highly significant difference equivalent to some twelve to fifteen times the standard error of a mean. This equal deviation of body size is accompanied, however, by a difference in larval period, since the large strains selected on the sub-optimal diets do not differ from unselected flies, while the strain selected on live yeast takes appreciably longer to develop.

(ii) Both strains selected on the sub-optimal diets respond in much the same way to progressive protein deficiency and the deviation from unselected tends to increase as protein level declines up to a maximum difference of about 30%, which is really quite striking. In the strain selected on the live yeast medium, the deviation of 0·14 on the log scale is reduced to 0·06 on 2% casein and virtually to zero (0·02) on the diluted medium, while development time is greatly prolonged (Table 4).

(iii) These contrasts originate naturally in the differing extent to which body size is reduced by the alternative diets and it is instructive to look at the data from this angle. Accordingly, the deviations from body size on the live yeast medium are set out in Table 5. The two strains selected on sub-optimal diets show no decline in body size until the casein level falls to 3%, while the maximum decline at the lowest level used is about 15%. The strain selected on live yeast resembles the unselected in showing greater proportional decline at higher protein concentrations, but differs by being more adversely affected when the casein level falls to 2% or the medium is diluted; in the latter case while the unselected show a 30% decline, the large strain is 40% smaller.

Table 5. *Reduction in body size below the level on live yeast—log scale*

	Genotypes according to diet during selection			
Medium	Unselected	Low protein	Diluted	Live yeast
6% casein	−0·07	0·02	0·00	−0·10
5% casein	−0·05	0·00	0·01	−0·07
4% casein	−0·10	0·02	0·01	−0·17
3% casein	−0·18	−0·04	−0·05	−0·18
2% casein	−0·30	−0·14	−0·17	−0·37
Dilute	−0·29	−0·14	−0·15	−0·41

Thus, the earlier evidence, after six generations of selection, that the large strain suffered relatively greater proportional decline on a low-protein diet, is reinforced by this test carried out after a further generation of selection. Body size of the strains selected on sub-optimal diets is only slightly greater on 6, 5 and 4% casein than on live yeast, compared with the earlier test in which the difference was greater. Such differences may be due to variation in the composition of the live yeast medium in successive tests.

(iii) *The comparison of strains under competitive conditions*

Ideally we should like to relate the behaviour of the large strains selected on different diets to which they have become more or less adapted to the kind of sub-optimal conditions encountered in nature or population cage. One approach to this problem is to grow larvae at different levels of competition in the ordinary medium. This was done in the following way. The ordinary maize-meal molasses medium, unfortified with dried yeast, was poured into small vials (diameter 1·7 cm.), to a depth of approximately 1 cm.; only vials with apparently the same volume of medium were used. Different levels of food supply and competition were established by setting up different numbers of newly emerged larvae. To provide a check on the general homogeneity or otherwise of conditions, larvae carrying the dominant *Bar* were set up along with the unselected or one or other of the three large strains, so that each culture was started with an equal number of *Bar* and non-*Bar* larvae. The *Bar* strain had been created by backcrossing into the *Pacific* wild population for a couple of years, so we can regard the marked flies as genetically equivalent to the wild stock apart from, at most, a small region on the X chromosome in the immediate vicinity of *Bar*. The levels of crowding were 20, 40 and 50 total larvae per tube—divided equally between marked and unmarked flies respectively—and 10, 5 and 3 replicates for the three levels of crowding respectively, were set up for each of the four strains. Development time was recorded for all flies which hatched and all flies of both sexes were measured. Complete variance analysis has been carried out for the extensive data, but these are not quoted since their chief value lies in the provision of error variances for comparing averages.

The first question to be asked is whether the *Bar* flies can be regarded as entirely equivalent to unmarked wild individuals with respect to survival under these conditions. The answer is given in the left-hand column of survival values in Table 6, which shows differences in the percentage survival of *Bar* and non-*Bar* individuals. In competition with the unselected, unmarked flies, *Bar* individuals survive as well at all levels of crowding. The average difference in percentage survival is negligible. Also progressive increase of the level of crowding from twenty to fifty larvae per tube is virtually without effect on the survival of either marked or unmarked unselected flies, and the mean value is a little over 70%. Hence the survival of *Bar* flies, when competing with other strains, can be taken as a suitable measure of how the unmarked, unselected individuals would behave in these circumstances.

When the marked individuals compete with the selected strains, we find a considerably higher level of survival when competing with the strain selected on low protein, about the same value with the strain selected on dilute medium, and a considerably higher level in competition with the large live yeast strain, except where fifty larvae are present—a discrepancy which will be referred to later. The middle section of Table 6 shows the differences in percentage survival between the marked individuals and the various strains with which they are competing. Since, as far as survival is concerned, we have concluded that marked and unmarked flies

Table 6. *Survival at different levels of crowding*

Percentage survival of marked controls in presence of different strains

Larvae per culture	Large strains according to diet during selection			
	Unselected	Low protein	Diluted	Live yeast
20	74·2	99·0	72·0	80·0
40	70·5	79·0	77·0	82·0
50	71·3	78·5	68·0	60·6
	Difference from controls: percentage survival			
20	4·0	−29·0	1·0	−22·0
40	−1·0	−20·0	−14·0	−22·0
50	0·0	−25·2	−27·0	−0·7
	Average total flies per culture			
20	14	17	14	14
40	28	28	28	29
50	36	33	33	25

are equivalent, we may infer that all the selected large strains have lowered ability to survive under these conditions of limited food supply and competition, compared with the unselected individuals, but differ somewhat with respect to relative performance at different levels of crowding. Thus, the large strain, selected on the dilute medium, survives as well as unselected flies when the cultures are started with twenty larvae, but competes less effectively when the number is increased to forty and especially fifty. The apparent increase in survival of the large, live yeast, strain at fifty larvae per tube, is almost certainly an artifact; probably too few *Bar* larvae were set up in these cultures in error.

The lowest section of Table 6 lists the average total number of flies, irrespective of genotype, which hatch from the tubes at different levels of crowding. For any level, the total hatch appears to be independent of genetic composition, so that the more vigorous, marked, wild individuals must supplant their less efficient competitors; it would be hard to find a more elegant demonstration of competition which discriminates between alternative genotypes.

The next question is whether or not the *Bar* flies differ in body size according to the strain with which they are competing. Table 7 whows the mean body size of the marked females in the different situations. It turns out, for any level of

Table 7. *Mean body size of marked, unselected females cultured in the presence of other strains—*$3 \times$ *log thorax length*

Larvae per culture	Large strains according to diet during selection				
	Unselected	Low protein	Diluted	Live yeast	Average
20	13·76	13·69	13·76	13·72	13·73
40	13·59	13·55	13·57	13·57	13·57
50	13·46	13·50	13·49	13·41	13·47
Average	13·60	13·58	13·61	13·57	

crowding, that the average body size of marked flies, does not differ according to which of the other strains is present; the variance analysis indicates that, for any row of values, the differences between means fall well within the range of error variances. The averages, quoted in the end column, show that an increase from twenty to fifty larvae per culture involves a decline of approximately 25% in body size. Since the average body size is comparatively constant for a given level of crowding, the performance of the various strains can be compared by reference to that of the marked flies whose behaviour can be regarded as typical of unselected flies. Table 8 lists the comparisons in terms of deviations of the values for body size and development time, at different crowding levels, from the values recorded for the unselected, while Table 9 sets out the comparisons as deviations from the performance on the uncrowded, live yeast medium.

Under the competitive conditions on the live yeast medium the large strains differ clearly in behaviour in spite of their outward resemblance when grown on the favourable live yeast medium. Thus, in the low-protein strain, crowding at twenty larvae per tube reduced body size below the level found on live yeast by some 50% compared with only 30% for the unselected individuals (Table 8). The relative difference was somewhat diminished at fifty larvae per tube since the large strain suffered about 12% greater decline than the unselected.

Table 8. *The effects of crowding on body size* (S) *and larval period* (L) *compared with uncrowded, favourable conditions; log scale*

	Large strains selected on different media							
	Unselected controls		Low protein		Diluted		Live yeast	
Larvae per culture	S	L	S	L	S	L	S	L
20	−0·28	0·29	−0·48	0·36	−0·31	0·39	−0·29	0·34
40	−0·43	0·45	−0·55	0·50	−0·63	0·57	−0·49	0·51
50	−0·53	0·57	−0·65	0·64	−0·59	0·63	−0·68	0·93

The strain selected on the dilute medium resembles the low-protein strain at higher levels of crowding but at twenty larvae per tube it does not differ appreciably from the unselected in proportional decline of body size. The other large strain, selected on the live yeast medium, shows the same proportional decline at twenty larvae per tube, somewhat greater decline at forty while at fifty larvae per tube this strain shows the greatest decline and also a greatly lengthened development time.

Table 9 sets out the data from the other point of view, i.e. in terms of the deviation from unselected under alternative conditions. Clearly the phenotypic difference between selected and unselected is greatest when conditions are favourable and substantially less under competitive conditions. For the two strains selected on aseptic media this situation offers a striking contrast to similar comparisons on different kinds of sub-optimal diet, i.e. protein deficient and dilute

Table 9. *Deviations from unselected of different large strains at various levels of crowding—body size* (S) *and larval period* (L) *on log scale*

	Large strains according to diet during selection					
	Low protein		Diluted		Live yeast	
Conditions	S	L	S	L	S	L
Optimum	0·14	0·02	0·17	0·01	0·14	0·07
Crowded twenty larvae	0·05	0·09	0·17	0·11	0·16	0·12
Crowded forty larvae	0·05	0·07	0·07	0·12	0·11	0·12
Crowded fifty larvae	0·06	0·09	0·06	0·05	0·03	0·42

media which lead to increased difference in size between selected and unselected as the diet becomes increasingly sub-optimal (Table 5).

The development time of the selected strains consistently exceeds that of the unselected flies when compared under competitive conditions. For any strain, the

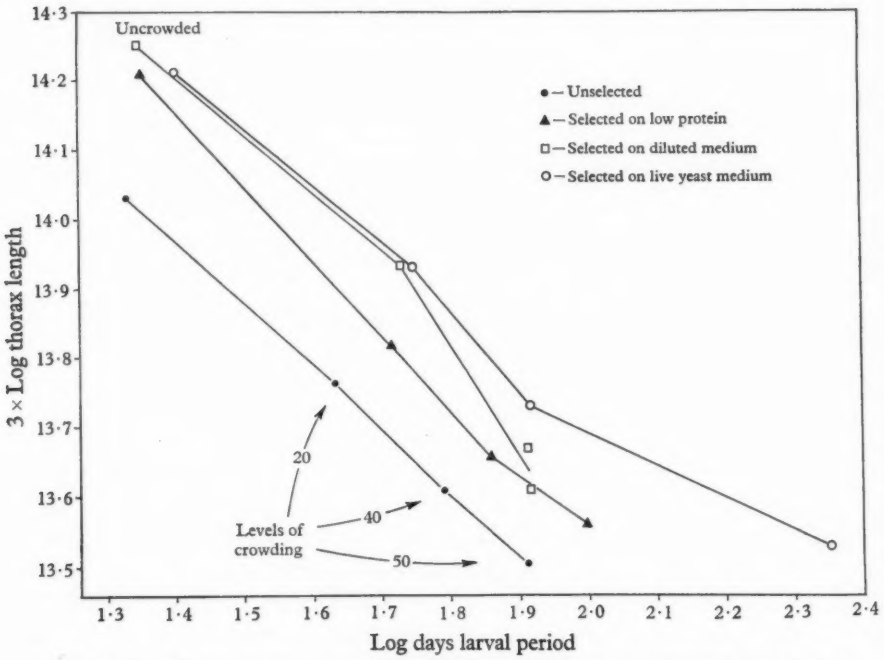

Fig. 4. Body size and development time of the unselected population and three large strains selected on different media, when grown in competition with genetically marked controls on the live yeast medium.

inverse relation between decline in body size and lengthening in development time is especially striking (Table 9 and Fig. 4). The only departure from the regular pattern occurs in the large strain selected on live yeast, where the larval period is disproportionately increased at fifty larvae per tube.

Now it so happens that the reduction of body size of the unselected flies below the level on the optimum live yeast medium is about identical when larvae are set up either in the small tubes—twenty per tube—or when they are grown on the synthetic medium with 2% casein or on the medium diluted to one-third strength. Comparing the performance of the various strains under these particular alternative conditions, throws into sharp relief their characteristic differences in behaviour. Table 10 shows the deviation of body size and larval period from the values found on the favourable live yeast medium.

Table 10. *Growth under sub-optimal conditions, expressed as deviations from the performance on the live yeast medium*

	Body size				Development time			
Treatment	U.	L.P.	D.	L.Y.	U.	L.P.	D.	L.Y.
Crowded, twenty larvae per culture	−0·28	−0·48	−0·31	−0·29	0·29	0·36	0·29	0·34
Diluted medium	−0·29	−0·14	−0·15	−0·41	0·46	0·47	0·53	0·58
2% casein medium	−0·30	−0·14	−0·14	−0·37	0·75	0·71	0·83	0·67

U., L.P., D. and L.Y. refer respectively to the unselected strain, and the large strains selected on low protein, diluted medium and live yeast. Body size is measured as $3 \times \log$ thorax length and development time as log days larval life.

Although the large strain which has been selected for large body size on the live yeast medium, suffers much greater decline in body size on the sub-optimal synthetic media, it is relatively much less affected than the low-protein strain by crowding at the level of twenty larvae per culture. Under such crowded competitive conditions, the unselected flies and the strains selected either on diluted media or on live yeast suffer about the same reduction in body size although development time is relatively longer in the selected strains. On the sub-optimal aseptic media, the strain selected on live yeast shows the greatest reduction and the other large strains least reduction in body size with the unselected roughly intermediate. These contrasts provide an excellent illustration of how selection under different nutritional conditions has altered the pattern of growth responses when larvae are grown on different diets.

4. DISCUSSION

These tests have amply confirmed the earlier inference that, on different diets, the variance of body size represents the effects of genetic segregation on different processes of growth and metabolism, according to how much and in what way the diets differ. Hence selection for the same 'character' in different conditions leads to qualitatively different changes. These have been revealed by the striking differences in reaction to similar alterations of diet on the part of strains selected on different media. Perhaps the most dramatic demonstration is embodied in Table 10, showing that three treatments which lead to the same proportional

decline of body size in unselected individuals—just under 30%—result in reductions of body size in the other strains ranging from 14 to nearly 50%.

Such contrasts are obviously related to the conditions under which selection has been carried out. Thus, when tested on the deficient media, both the unselected population and the large strain selected on live yeast medium suffer a much greater decline in body size than either of the large strains selected on the synthetic diets. Under the competitive conditions tested, which probably approximate more closely to the conditions in nature, the strain selected on the low-protein diet is much more reduced in size, while the live yeast strain suffers about the same decline as unselected flies, at intermediate levels of crowding, but a greater decline when the level of competition is increased. As might be expected, there are many different ways in which a diet can be rendered sub-optimal, with respect not only to the deficiency of specific essential nutrients, but also to the relative concentration of nutrients which may affect nutritional imbalance. It looks, on present evidence, as if the competitive conditions on the live yeast medium may be more like those commonly met with than either of the synthetic diets, although the composition of the diluted medium may correspond more closely to natural sub-optimal conditions than is the case for the low-protein diet. By testing the reaction of suitable genotypes to graded levels of competition, supplementing the medium with specific nutrients, it should be possible to determine the more important limiting factors in growth under crowded conditions on the live yeast medium and arrive thereby at a more realistic, controlled reproduction of the natural sub-optimal conditions.

The relatively superior performance on the deficient synthetic diets, of the strains selected under these conditions, it is entirely consistent with the other indications given in the earlier paper, that the larger body size in these strains is partly due to better adaptation to these conditions. It was suggested in the earlier discussion, that adaptation to such diets probably lead to some loss of adaptation to the ordinary live yeast medium. This inference is consistent with the relatively greater decline in body size when larvae are crowded on the live yeast medium. There is also the tendency, most evident in the low-protein strain, for body size to be slightly smaller on the normally favourable live yeast medium, than on Medium C, even when fructose is omitted.

The ease with which a few generations of mass selection can improve performance on particular sub-optimal media, implies the existence of freely segregating differences which can provide the means of immediate adjustment to a wide variety of different nutritional conditions, such as are commonly encountered in natural or laboratory conditions (Gordon & Sang, 1941). Since the reaction to differences in the chemical composition of the diet is genetically controlled and since the composition of the diet in nature is subject to wide fluctuations, there will be corresponding variation in selection pressures. Although gene arrays will be favoured, which, on the average, confer greatest independence of such variation in environment, there will be a limit to the effectiveness of such adaptation at the level of the individual. In an outbreeding species, the high level of heterozygosity provides

the material for an immediate adjustment to either short or long term changes in the environment. The readiness with which we can select for better adaptation to the special diets chosen for these experiments is probably related to the variety of conditions normally encountered by the species. It would be interesting to see whether differences between species in this respect could be correlated with the ecological diversity of their habits.

The relations between the two measures of growth—adult body size and duration of the larval period—must be considered next. Provided the larval diet is not too deficient, the larval period may be considerably extended without any reduction of body size below that attained under the most favourable conditions for growth. But there is a limit to this capacity for maintaining a constant body size and when the diet becomes too inadequate body size declines and this reduction is accompanied by progressive and more or less proportional lengthening of the larval period. The strains differ in their capacity to achieve maximum size in relation to a given change of diet. Thus on media which drastically reduced the body size of both the unselected population and the large strain selected on the live yeast medium, the size of strains previously selected on sub-optimal aseptic media was not reduced, although development time was increased. It appears that differences in the level of adaptation to different kinds of diet are correlated with differences in ability to maintain a constant adult body size. Also, different gene arrays selected under different nutritional conditions, may lead to the same maximum body size, but the range of conditions which allow constancy of final size may differ sharply. The increase in the variance of body size when the diet is altered sufficiently to reduce average body size below the maximum level (Robertson, 1960*a*) can be attributed to the exposure of previously undetected differences in this capacity to maintain body size.

As noted above, more adverse conditions which reduce adult size show a distinct relation between smaller size and longer larval period. Although all the tests on alternative media show evidence of this kind of response, perhaps the most striking example occurs in the comparisons of performance with different levels of crowding. Here, the regression of body size on larval period was very close to -1, on the log scale. Sub-optimal diets may differ in how far they involve disproportionate lengthening of the larval period compared with the reduction of body size. Thus Table 10 showed that crowding at twenty larvae per tube and also aseptic culture on either low-protein or diluted media cause the same percentage reduction of body size of the unselected population but give widely different development times. With crowding the development time was increased by about 30%—just as much as body size was reduced—while on the diluted and low-protein media, development time was increased by about 50 and 70%.

Such differing relations may depend on the stage of larval growth and development primarily affected by the treatment in question. There is evidence from tests carried out by Bakker (1959) and supported by my observations, that, after a certain stage early in the third instar, the subsequent duration of the larval stage is determined. This was shown by removing larvae from the food medium at

different ages. It appears that after this stage is reached larvae can pupate without further food and that variation in food intake will lead to great variation in adult size but little or no differences in duration of development. Nutritional effects of this sort could account for differences in size which are associated with the same development time, as noted in Fig. 1, for example. What remains to be determined, however, is the relation between diet, early larval growth rate, the critical period and constancy or otherwise of the larval period after the critical stage is reached. There is little point in speculating about possibilities until current experiments have clarified the situation. However, a few general observations are relevant.

The relations between body size and duration of the larval period are probably controlled by antagonistic reactions between hormones which favour continued growth in the larval stage, on the one hand, and the onset of differentiation on the other. To quote from a recent review by Karlson (1956): 'The holometabolous form of development results from the special balance between hormones from the corpora allata and those from the prothoracic gland during the larval period followed by a marked change in the balance during pupation and imaginal differentiation.' If a certain critical ratio of concentration of antagonistic hormones or alternately some critical threshold relationship has to be established to set an end to growth, relative constancy of such a ratio or threshold will make for a constant body size, other things being equal. Even though growth is slowed down, the critical state will be approached more slowly and will be reached only when the total amount of growth characteristic of the strain has been completed. Possibly some such situation underlies the maintenance of a characteristic body size.

But, as we have seen, there is a limit to the effectiveness of this maintenance, since body size declines when the diet is too deficient. This sharp change-over in the pattern of response suggests a characteristic change in the hormonal relations of the critical threshold. If the antagonistic relations or critical threshold were independent of the nature of the diet, development time would be indefinitely prolonged until the essential conditions were fulfilled. On the other hand, a change in hormonal relations or threshold can ensure that the adult reproductive state will be reached more quickly. To put it rather crudely, it may often be better for the species to be a small fly today, rather than a bigger fly tomorrow or a fully grown one next week. Hence, if the diet is too deficient a kind of safety mechanism comes into operation which alters either hormonal relations or critical threshold in such a way as to allow the adult state to be reached more quickly than would otherwise be the case.

The relation between the nature of the diet and the readiness to sacrifice body size and potential fecundity for a quicker approach to adult-hood, probably varies from species to species. For some species, the capacity to produce the maximum number of eggs—only possible if body-size is not reduced—may be relatively more important than variation in the length of the larval period, and, in such cases, the capacity for maintaining body size will be more highly developed than in others for which shorter life cycle is relatively more important than maxi-

mum egg production. Exactly where the balance is struck, in relation to the composition and quantity of the diet, will differ according to ecological conditions and the nature and intensity of intra- and inter-specific competition. Differences of this kind may well influence the course of selection for apparently the same 'character' in different species. For example, if there is a highly developed ability to maintain a characteristic body size when the diet is poor, response to selection for, say, larger size, might be expected to be greater than in another species which responds to sub-optimal conditions by reducing body size more readily. Hence observed differences in response to parallel selection may be essentially due to characteristically different inter-relations between genotype and ecology, rather than, say, differences in linkage relations or chromosome number.

In general, therefore, the evidence from this and the earlier papers in this series, implies that the physiological nature and magnitude of the response to selection for body size—and doubtless other attributes too—can be fully understood only by relating the particular conditions in which selection is practised to those prevailing in the normal environment.

SUMMARY

1. The growth of strains of *Drosophila melanogaster* selected for large size under different nutritional conditions has been recorded on a variety of different media and compared with that of the unselected population. The experiments were designed to test the inference from earlier work that selection for the same 'character', body size, on different diets leads to more or less different changes in growth and metabolism. The inference has been amply confirmed.

2. When compared on a number of deficient synthetic diets, the strains which had been selected either on a low-protein diet or on one in which all the essential nutrients had been reduced, suffered a much smaller reduction in body size than either the unselected population or, especially, a large strain selected on the favourable live yeast medium. Some diets which drastically reduced the body size of the unselected population lead to no change in the size of strains selected on the synthetic media, although development time was prolonged. Hence selection had extended the capacity for maintaining a characteristic adult body size to diets which normally would lead to a decline. This is taken as evidence of improved adaptation to such conditions. There is also some evidence that selection on the synthetic diets had lowered the level of adaptation to the usual live yeast diet, since body size tended to be lower on this medium than on some of the normally sub-optimal diets.

3. To provide comparisons in adverse conditions which are probably more closely related to those commonly encountered by populations in nature or the laboratory, the performance of the strains has been compared in a graded series of competitive conditions on the live yeast medium. By using genetically marked flies of the foundation population, which were shown to react in the same way as unmarked flies—in terms of survival, body size and development time—the competitive ability of the different strains has been tested against that of unselected

individuals. The latter are generally superior to the selected strains, which differ among themselves, however, in a way which can be related to the conditions in which they were selected.

4. Under such competitive conditions, the strains selected on the synthetic diets suffer a much greater decline in body size than do the unselected individuals. For the strain selected on live yeast, the proportional reduction of body size is about the same for the unselected flies at lower levels of crowding, but is clearly greater under more severe conditions of competition.

5. The low-protein strain has been backcrossed to the unselected stock. When reared on a variety of synthetic diets, the performance of the F_1 was generally intermediate between that of the parents.

6. Nutritional variation may be responsible for either a high environmental correlation between the two measures of growth, body size and duration of larval period, or no apparent correlation. Provided the diet is not too unfavourable, body size remains constant although development time may be lengthened to a variable degree. With more adverse conditions, body size is reduced and development time is lengthened more or less proportionately. Such differences in reaction probably depend on the particular stage of larval growth and development primarily affected by the treatment; this problem is being examined further. The inverse relations between body size and development time may represent the operation of a kind of safety mechanism which ensures that the adult reproductive state is attained sooner than would be so if the capacity for maintaining a characteristic body size were more effective in relation to deficient diets. Populations and species adapted to different conditions are likely to differ as to where the balance is struck between effective maintenance of a characteristic adult size, with maximum potential egg production, and the alternative response, according to their ecology. This possibility must be borne in mind when the response to selection for, say, body size is compared in different species.

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The genetic sensitivity to X-rays of mouse foetal gonads

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Estimates of the dose to human gonads of man-made radiation show that nearly all of it comes from radiation used in medicine, rather than from fall-out or atomic energy, and that of the medical dose approximately one-third is delivered to post-natal males, one-third to post-natal females and one-third to foetuses (Medical Research Council, 1956). Therefore in investigating the genetic hazard to man of ionizing radiation it is important to have some measure of the sensitivity of foetal germ-cells to radiation mutagenesis. Carter (1958) irradiated foetal male mice with 300 r. X-rays at 13½ days' gestation and tested them, as adults, for mutations at seven specific loci. He found only one mutant among 10,155 progeny tested and put forward the tentative conclusion that 'the yield of induced mutation, as recovered from spermatozoa of the adult, is lower for irradiation of foetal than of adult spermatogonia'.

The present paper describes further experiments on foetal germ-cells, designed to amplify this work.

Carter irradiated foetuses at 13½ days' gestation, when the gonad is still populated by primordial germ-cells and sex-cords are just developing. This corresponds to a stage of about 5 weeks' gestation in human embryos (Otis & Brent, 1954). But the greater part of the dose of medical radiation to human foetuses is delivered in late pregnancy. Therefore, in extending the work on mouse foetuses it was of interest to irradiate at a later rather than an earlier stage of gestation. The stage chosen was 17½ days, being the latest convenient stage, and the offspring of both sexes of foetuses were examined for mutations. At this stage female germ-cells are in the early stages of meiotic prophase (Brambell, 1927), and male germ-cells are still in the form of primordial germ-cells. This corresponds to a stage of about 5 months' gestation in human foetuses (Maximow & Bloom, 1952). Rugh & Jackson (1958) showed that irradiation of foetal mice may result in permanent sterility and that the dose which will cause sterility varies greatly between the sexes and among different foetal stages. The dose, 200 r. X-rays, used in the present experiment was the highest consistent with fertility of both sexes when given at 17½ days' gestation.

STOCKS AND METHODS

Female mice of the C3H/HeH strain were placed with 101/H males and examined daily for copulation plugs. Seventeen days after the finding of a plug they were given a dose of 200 r. X-rays (250 kV, 14 mA, HVL 1·2 mm. Cu, 72 r./min.) to the

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whole body from below. The young born to them, which were C3H × 101 hybrids, were mated when about 8 weeks old with animals homozygous for the seven recessive genes, *a*, *b*, c^{ch}, *d*, *p*, *s*, and *se*. The young born to these pairs were examined when $2\frac{1}{2}$ weeks old for mutations at these seven loci and for dominant visible mutations at any loci. A target of 30,000 progeny was set for each sex.

RESULTS

The animals irradiated as foetuses were smaller than unirradiated animals of the same genotype, but no congenital malformations were noticed among them, and their mortality in the nest was not unduly high, considering their small size. Altogether 621 males were mated, of which 47, or 7·6%, proved to be sterile and a few sired fewer young than normal, and 725 females of which only 4 were sterile.

Table 1 shows that among the progeny of irradiated females there were three specific locus mutants and one dominant visible mutant, and among the progeny of males there were nine specific locus mutants and five dominant visibles.

Table 1. *Mutant animals found*

Sex irradiated	Total progeny	Specific locus mutants							Total	Dominant visibles
		a	*b*	*c*	*d*	*se*	*p*	*s*		
♀	30,289	–	1	1	–	–	1	–	3	1
♂	31,253	–	1	2	1	(1)	1	4	9	5

All the specific locus mutants were genetically tested and found to carry mutant alleles at the loci shown. One, in the male series, carried mutant alleles at both of the very closely linked loci *d* and *se*. In the analysis of the results this has been counted as a single mutational event. Three of the four mutants at the *s*-locus occurred in two litters from the same irradiated male; these have been counted as three mutants.

The dominant visible mutation in the female series caused circling behaviour. Those in the male series were two in which heterozygotes resembled those of the *W*-series; one in which heterozygotes and homozygotes phenotypically resembled splotch, *Sp*; a sex-linked mutant resembling tabby, *Ta*; and a short-tailed mutant.

COMPARISON OF FOETAL AND ADULT SENSITIVITY TO MUTAGENESIS

Russell, Russell & Kelly (1958) showed that when adult male mice are given a dose of 300 r. or less of X-rays and young are raised from treated spermatogonia the average mutation rate at the same seven loci as were used in the present experiment is about 28×10^{-8}/r./locus. Russell, Russell & Cupp (1959) showed that the mutation rate after similar treatment of females did not differ from that obtained after irradiating males. Therefore, to test whether the sensitivity to mutagenesis of the foetuses in the present experiment differed from the sensitivity of adults, the numbers of mutants observed have been compared with the numbers that would have been expected had the induced mutation rate been 28×10^{-8}/r./locus (Table 2).

Since the comparison is being restricted to the same seven loci throughout and there is no extrapolation to mouse loci in general, the confidence limits given by the Poisson distribution have been used (Fisher & Yates, 1953, Table VIIIi), rather than the wider confidence limits suggested by Kimball (1956) for use when arguing from species to species. In calculating the expected number for the male series allowance has been made for an expected 1·9 spontaneous mutants. This estimate is based on the combined figures for male spontaneous mutation of Russell, Russell & Kelly (1958) and Carter, Lyon & Phillips (1958), which were 25 mutants out of 406,343 animals, giving an average mutation rate of $0{\cdot}85 \times 10^{-5}$ per locus. The spontaneous mutation rate in the female mouse is at present unknown since Russell, Russell & Cupp (1959) found no spontaneous mutations among 46,763 offspring of untreated female mice. Therefore, since the number of mice raised in the female series here was less than 46,763 the expected number of spontaneous mutations has been taken as zero. If this should prove to be an error then the expected number of mutants here would be greater, and the significance of the results would be increased.

Table 2. *Comparison of observed mutants with numbers expected on the basis of the adult mutation rate*

Sex irradiated	Total progeny	Mutants expected	Mutants observed	Confidence limits*		Observed induced mutation rate/r./locus $\times 10^8$
♀	30,289	11·9	3	0·619	8·77	7·1
♂	31,253	14·2	9	4·12	17·08	16·2

* Lower and upper 95% confidence limits of observed number. (Fisher and Yates, 1953, Table VIII.)

Table 2 shows that for both the male and female foetuses the observed number of mutants was below the expected number. For the females, but not for the males, the expected number was outside the 95% confidence limits of the observed number. However, the expected number was itself based on experimental data and therefore had its own confidence limits. The actual figures obtained by Russell, Russell & Cupp (1959) after giving 400 r. X-rays to adult females were 7 mutants out of 7,859 offspring examined, giving a mutation rate of $31{\cdot}8 \times 10^{-8}$/r./locus. The probability of obtaining by chance a difference as great or greater than that between the mutation rates per roentgen for these two groups of female mice was $P = 0{\cdot}023$ for a one-tailed test. Such a test has not been made for the males since the observed and expected figures differed so little.

In the case of the male foetuses we may also make another comparison: between the mutation rate obtained in this experiment using the $17\frac{1}{2}$-day stage and that found by Carter (1958) using the $13\frac{1}{2}$-day stage. Table 3 shows this comparison. The mutation rate per locus per roentgen at the $13\frac{1}{2}$-day stage was only about one-tenth of that at $17\frac{1}{2}$ days. However, the probability of finding a difference as great or greater by chance is as high as $P \sim 0{\cdot}1$ for a one-tailed test.

Table 3. *Comparison of results from male foetuses of 13½ and 17½ days. The adult figures are those of Russell, Russell & Kelly* (1958)

Stage (days)	Dose (r.)	Total progeny	Mutants	Mutation rate $\times 10^5$	Induced mutation rate/r./locus $\times 10^8$
13½	300	10,155	1	9·85	1·76
17½	200	31,253	9	28·8	16·2
Adult	300	40,408	25	61·9	28

DISCUSSION

In discussing the results of this experiment one must first consider that it has been necessary to make comparisons between experiments carried out in different laboratories using different equipment and stocks of mice. The mouse stocks used at Harwell were branches of the stocks used at Oak Ridge, and Phillips (1960) found no evidence of any gross difference in sensitivity of adult males of the two branches to the mutagenic action of acute and chronic doses of 600 r. Therefore it may be concluded that differences in the stocks and experimental techniques do not account for the differences between the Harwell and Oak Ridge results that are discussed here.

The results of this experiment indicate that when female mice were irradiated as 17½-day-old foetuses the observed mutation rate among their offspring was lower by a factor of about four than after similar irradiation of adults. When the irradiated 17½-day-old foetuses were male mice the observed mutation rate was below but not statistically significantly below the comparable adult rate, but neither did it differ significantly from the rate for 13½-day-old male foetuses, which Carter found to give a lower mutation rate than adults.

There are two main possible explanations for the differences found: (1) that the rates of induction of mutation were different in the various life-stages irradiated, and (2) that the rates of induction were constant but that differing amounts of elimination of mutant cells by selection during gametogenesis affected the rate of discovery of mutants. Either of these explanations could be the real one. Russell, Bangham & Gower (1958) showed that in adult male mice the mutation rate varies with the germ-cell stage irradiated. In foetal gonads the germ-cell stage attained is different from that in adults. In 17½-day-old foetuses the female germ-cells are in early stages of meiotic prophase whereas in adults they are resting in the dictyate stage at the end of prophase; in the male gonad the primordial germ-cells are still present at 17½ days whereas the results from adults which have been quoted refer to spermatogonia. Thus it is possible that the rate of induction of mutation in foetuses differs from that in adults as a result of the difference in germ-cell stage irradiated. On the other hand, selective elimination of mutant cells might also occur, particularly in males. In this experiment about 7·6% of males were sterilized by the radiation dose they received. This suggests that the cell-killing effect of the radiation on the germ-cells was severe, and that the testes were repopulated from a

very small number of surviving cells. If there were any differential sensitivity the observed mutation rate among the surviving cells might be spuriously low.

Thus this experiment, though amplifying Carter's earlier work, still leaves much unknown about the genetic effects of foetal irradiation. Though female foetal mice of the stage used seem clearly less susceptible than adults, this is not certain for males. It seems reasonable to expect that different foetal stages will vary in their sensitivity to mutagenesis, but the data are not so far adequate to establish this. From the point of view of human hazards, however, it is reassuring that there is no indication so far that any age or sex of foetus is more susceptible than the adult male.

SUMMARY

The mutation rate at seven specific loci was measured among the offspring of male and female mice exposed as $17\frac{1}{2}$-day-old foetuses to 200 r. X-rays. In the female series the mutation rate was lower, by a factor of about four, than the comparable adult rate; in the male series the mutation rate was lower but not statistically significantly lower than in adults.

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Forward and back mutation in the *pyr3* region of Neurospora. I. Mutations from arginine dependence to prototrophy*

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1. INTRODUCTION

An important limitation of most screening systems available for mutation studies is that they allow selection of the mutations in one direction only, e.g. from nutritional deficiency to prototrophy, from inactive to active allele. The recent work of Freese (1959) on bacteriophage has strikingly vindicated the importance of working with systems which permit the selection of mutants in both directions (forward and back) at a single locus. As pointed out by Westergaard (1960), it is desirable that systems of this kind be established in organisms of different degrees of complexity. Moreover, it is desirable to be able to screen not only for the extreme phenotypes, but also for the widest possible range of intermediate mutant phenotypes.

With such considerations in mind, we have been studying the behaviour of a system for screening forward and back mutations at the *pyr3* (*pyr*) locus of Neurospora (Reissig, 1958, 1959). Selection for forward mutations at the *pyr* locus is possible since *pyr* suppresses a certain arginine mutant, *arg2* (*arg*); i.e. the double mutant *arg pyr* is pyrimidine-dependent, but arginine-independent. On plating uninucleate conidia of the *arg* strain on a medium supplemented with pyrimidine, but lacking arginine, two phenotypically distinct classes of mutants are obtained: pyrimidine-dependent mutants of the *arg pyr* constitution (I in Fig. 1), and prototrophs. The back-mutation pattern of the *pyr* mutants can be studied by the usual back-mutation technique (II in Fig. 1).

The present paper describes the prototrophs obtained after treating a macroconidial *arg* strain with u.v., and plating on a medium free of arginine or pyrimidines. As will be shown, such reversions can result from any of the following events (Fig. 1): back mutation at *arg* (III); forward mutation from pyr^+ to *pyr* (I), followed by heterokaryon formation; or forward mutation from pyr^+ to $pyr^{su\text{-}arg}$ (IV).

2. MATERIALS AND METHODS

Strains

The arginine-dependent strain carries the *arg2* (*arg*) allele, isolated as 33442 by Beadle & Tatum (1945) after u.v. treatment. As *arg* can utilize citrulline as well as

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arginine for growth, this allele has been occasionally referred to as *cit* (Mitchell & Mitchell, 1952; Reissig, 1958, 1959). Other mutant alleles used are: *pyr*, obtained by spontaneous mutation (Reissig, 1958) and allelic to the *pyr3* gene isolated as 37301 (Mitchell & Mitchell, 1956; Reissig, unpublished); *colonial-4* (*co*), isolated as 70007 (Mitchell & Mitchell, 1954); and *cot*, isolated as C102 (Mitchell & Mitchell,

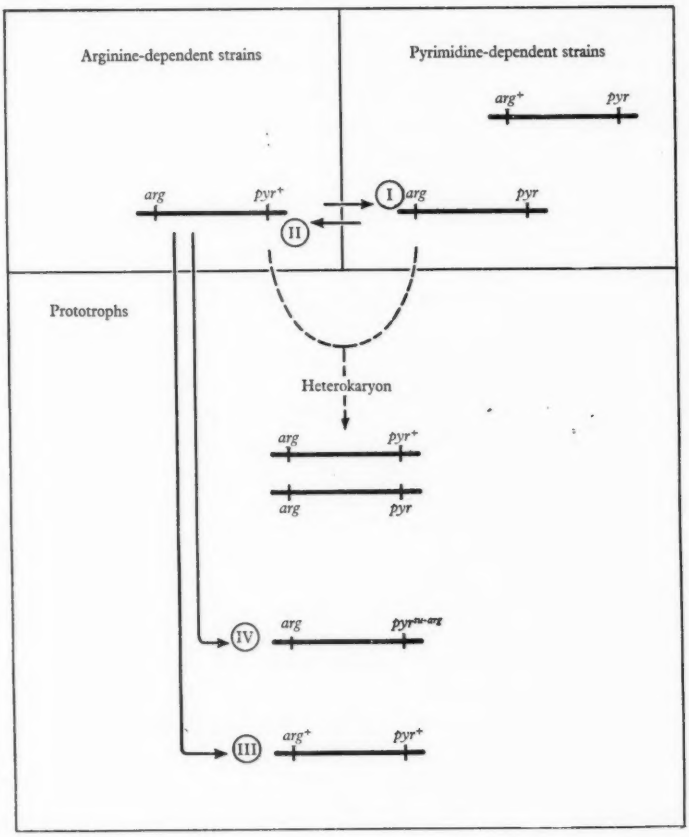

Fig. 1. Mutations at the *pyr* and *arg* locus which are readily obtained by screening methods reported in this study. The solid arrows indicate mutational steps. The broken arrow indicates the composition of the heterokaryon.

1954). The latter two mutants determine colonial growth: *cot* gives small colonies at 32° C., but spreading growth at 25° C.; while *co* grows colonially at all temperatures. The *cot co* and *cot co*$^+$ strains are easily distinguished from each other by culturing at 32° C., cooling to 25° C., and some hours later examining the halos which form around the small colonies. Intermediate colonial phenotypes, such as those obtained in pseudo-wild types (Mitchell, Pittenger & Mitchell, 1952), can also be identified by this method, but not with certainty.

All the above markers are linked (Mitchell & Mitchell, 1954). The reported map distances are: centromere–13–*co*–0·4–*arg*–1·1–*pyr*–25–*cot*, on chromosome IV.

Strain ED38-35a was used in all mutation experiments. It is macroconidial, and carries the *arg*, *co* and *cot* markers. The *cot* allele was present in all the strains used.

Culture media and temperature

The M/15 phosphate medium of Strauss (1951) was arbitrarily chosen for the screening of mutants. It was adjusted to pH 4·5, and supplemented with 1 gm./l. of ammonium sulphate. The salt solution was autoclaved separately from the other ingredients. Crosses were performed on the medium of Westergaard & Mitchell (1947). In all other cases, the solid medium of Fries (Beadle & Tatum, 1945) was used.

All cultures were grown at 25° C. In order to obtain colonial growth, all plates were incubated at 32° C., unless otherwise indicated.

Supplements

L-Arginine HCl and L-lysine mono HCl were obtained from Light and Co. The L-canavanine sulphate was prepared from jack beans (*Canavalia ensiformis*) by the method of N. H. Horowitz and M. Fling (personal communication). Hydrolysed RNA was prepared by the following method. A sample of yeast nucleic acid (The British Drug Houses, Ltd.) was divided into two equal portions. These portions were dissolved in N HCl and N NaOH, respectively, heated for 20 minutes at 100° C., mixed, neutralized, and filtered while hot. Hydrolysed RNA was added at a concentration of 0·5 to 1 g. of nucleic acid per litre of medium. Arginine was added at 0·05 to 0·1 g./l. for vegetative cultures, and 0·4 g./l. for crosses.

Production and screening of revertants

Strain ED38-35a was grown for 2–4 weeks in flasks with 50 ml. of arginine-supplemented medium. The conidia were harvested in water, filtered through cotton wool and washed three times in water by centrifugation. A portion of this suspension was irradiated with u.v. light. The control series was handled in a similar fashion, with the exception that the irradiation step was omitted. Two alternative procedures were used for irradiation. In one, the conidial suspension was exposed to u.v. while being magnetically agitated, washed once by centrifugation, diluted as required and plated at concentrations ranging from 5×10^5 to 3×10^6 conidia per plate. In the second procedure, the conidia were first plated and then irradiated within 2 hours of plating. Since the sensitivity of the conidia to u.v. increases significantly in the first few hours after plating (Reissig, 1960), the latter procedure cannot be relied upon for quantitative work.

The u.v. source was either a Philips germicidal tube TUV 15 W, or a Hanovia Germicidal Unit. In both cases most of the output of the source was at 254 mμ. All operations during and after irradiation were performed under low-intensity yellow light.

The plates for the screening of mutants were poured in three successive layers. The bottom layer consisted of 12 ml. of minimal medium. If the irradiation was to be performed on the plates, the conidial suspension was spread on top of this layer. If the conidia had been irradiated in water, they were mixed with 3 ml. of minimal medium kept liquid at 42° C., and poured as a second layer. This second layer was also added on top of the conidia after irradiation on the plates. The *arg* mutant is a leaky one; i.e. it can grow slowly in the absence of arginine. This slow growth can be inhibited by canavanine. Further supplementation with lysine protects revertant clones from partial inhibition by canavanine. Therefore, a third layer (LC) of medium supplemented with 0·05 g./l. of lysine, and 0·15 g./l. of canavanine, was added on top of the previous two layers. Unless otherwise specified, LC was added 9–12 hours after plating (cf. section 3—I).

On the fourth day the plates were cooled to 25° C. for about 7 hours. In this way the revertant colonies spread out, permitting a better differentiation between the fast-growing revertants and background clumps. The plates were scored on the fifth day. Viability was scored in a similar fashion, adding an arginine-supplemented overlayer instead of LC.

Some of the above-specified conditions for the detection of revertants are critical, and appreciable departures from them may affect the residual growth of non-mutant conidia, and the recovery of mutants. Less growth, and in general fewer mutants, are obtained by using older cultures, lower conidial densities, more canavanine, less lysine, or by adding LC at an earlier time.

It is not possible to know whether all types of revertants are recovered; indeed, there could be types which completely escape identification under the conditions employed. We believe, however, that the recovery is essentially complete for the (major) types of revertant being scored. Evidence for this comes from reconstruction experiments with four different revertants, and from the fact that a plateau in the yield of revertants is reached when plating density is increased or when addition of LC is delayed.

The effect of plating density on recovery alerted us to the possibility of falling into the pitfalls described by Grigg (1952) (see also Kølmark & Westergaard, 1952). We tested for plating interactions by two methods (Srb, 1958); plating varying dilutions of treated conidia, and plating mixtures of treated and untreated conidia. The only effect detected was a drop in the recovery of revertants in plates containing less than approximately 5×10^5 conidia. While this effect may lead to an underestimate of the reversion frequency under conditions of low survival, it points reassuringly in the opposite direction from the 'Grigg effect'.

Analysis of crosses

Ascospores were washed by centrifugation, first with sodium hypochlorite solution (*ca.* 0·7% of available chlorine), and then with water, thereby killing all vegetative cells. The suspension was then heat-activated and plated in the usual way.

The detection of infrequent arginine-dependent segregants among a population of arginine-independent colonies was accomplished by the use of replica-plating

(Reissig, 1956). In this method minimal plates are covered with discs of cigarette paper ('pipe-papers' from Rizla Cigarette Papers, Ltd., Wembley, England). The ascospores are spread on top of the paper at a density of *ca.* 100 viable spores per plate, and incubated until the colonies grow into the agar. The paper is then lifted with forceps and transferred to an arginine-supplemented plate. Colonies developing on the second plate only are either slow growers (*ca.* 1% of the population in our crosses) or arginine-dependent. The recovery of arginine-dependent colonies is affected by the extent to which the agar surface is covered with prototrophic colonies. Reconstruction plates were set up with each experiment. In such a plate, an aliquot of the population being assayed and an aliquot of a population of arginine-dependent ascospores were inoculated simultaneously. The concentration of spores in the latter population was determined by plating on arginine media. This value can then be compared with that obtained from the reconstruction plates, the ratio of the two being called 'recovery ratio'. Whenever justified, recovery ratios from simultaneous experiments were pooled for tabulation.

Testing for nutritional requirements (spot-test)

This was done by depositing with a loop the conidial suspensions to be tested on the surface of plates with the discriminating media. The plates were scored after 3 days. The revertant phenotype was scored on plates with and without arginine.

3. RESULTS

I. *Expression of the u.v.-induced revertants*

Delay in the occurrence of a mutation or in its expression is known to take place in several systems. If this delay occurs in a system for selecting drug-resistant mutants, the resistant clones would fail to grow up if the drug is added immediately after the treatment. This is precisely what is observed in the present system: the maximum yield of mutants is realized only if the cells are incubated for about 9 hours after plating and before adding LC. Several mechanisms could be responsible for this behaviour:

(*a*) Lag between the primary effect of u.v. and the occurrence of the mutation (delayed mutation).
(*b*) Lag in the phenotypic expression of the newly induced mutation (delayed phenotypic expression).
(*c*) If the resistant trait is partially recessive, a delay in the establishment of the favourable ratio of mutant and wild-type nuclei in the heterokaryon (selection delay).
(*d*) Physiological factors independent of the time when mutation was induced, e.g. the drug prevents conidial germination in the resistant strains (physiological lag).

Mechanisms (*a*) and (*b*) operate only with newly induced mutants, while (*c*) could, and (*d*) would operate regardless of the time when the mutation was induced. It

is therefore possible to decide between (*a*) and (*b*) on the one hand, and (*c*) and (*d*) on the other, by comparing the behaviour of newly-induced mutants with that of a random sample of established mutants.

A population of *arg* conidia was plated on minimal medium, irradiated, and transferred 8 hours later to a culture flask supplemented with arginine. This culture was allowed to grow and conidiate. The proportion of revertants among the conidia formed in this flask was *ca.* 0·2%. This is higher by two orders of magnitude than is

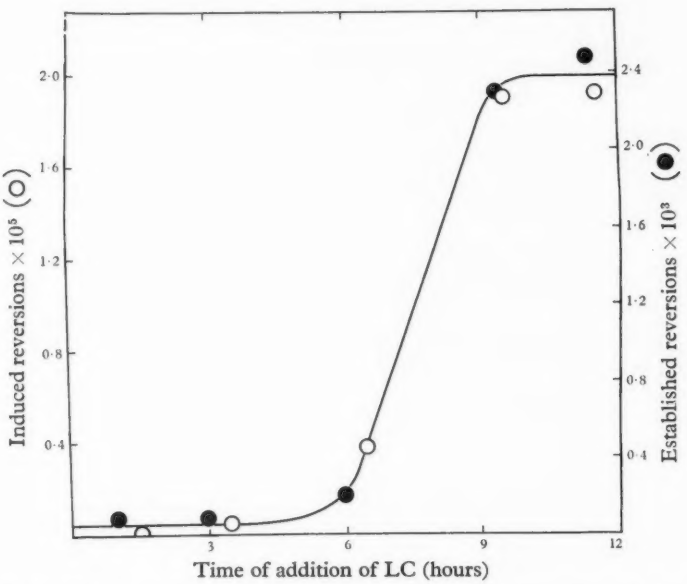

Fig. 2. Lag in the appearance of revertant colonies. The experimental procedure is described in the text. Time zero is the time of plating. Each point represents three to six plates. Closed circles, established reversions; open circles, newly induced reversions.

usually observed when scoring directly after irradiation. The difference was probably due to selection during growth. The high frequency of established mutants encountered permitted further irradiation of the conidial population without causing a significant increase in the number of revertants present. In two parallel series, conidia from the above culture and from an untreated *arg* culture were harvested, irradiated in water, and plated at concentrations of 10^5 and 10^6 per plate, respectively. The former series reflects the behaviour of established mutants, the latter the behaviour of newly-induced mutants. The results (Fig. 2) show that the time-dependence for the appearance of mutants is the same for both series, thus indicating that mechanisms (*a*) and (*b*) are either excluded or of minor importance.

Similar experiments were also performed with microconidial strains. The time of addition of LC again had a pronounced effect on the yield of mutants. In

this case only mechanism (*d*) could be operating since the microconidia are uninucleate.

The timing of the physiological lag is correlated with conidial germination. In three experiments, the beginning of germination coincided with the time when addition of LC allowed the first clear increase in the yield of mutants. Over 50% of the conidia had germinated when the plateau was reached, supporting the hypothesis that the lag is due to interference of LC with conidial germination.

Granted that the physiological lag is the major cause of the observations depicted in Fig. 2, the evidence does not exclude the occurrence of delayed mutation, delayed phenotypic expression or selection delay as minor components of the observed phenomenon. The following experiment was designed as a more sensitive test for such effects.

Untreated *arg* spores were plated, and 9 or 11 hours later overlayered with LC. Irradiation was performed at varying times after plating and before overlayering. An untreated control series was also run. The results shown in Table 1 indicate

Table 1. *Testing for mutational delay*

Time of irradiation	Revertants per total conidia plated. Time of overlayering		Revertants per survivor $\times 10^5$. Time of overlayering	
(hrs.)	9 hrs.	11 hrs.	9 hrs.	11 hrs.
2	124	138	2·6	2·9
$6\frac{1}{2}$	97	95	2·4	2·4
9	115	102	3·5	3·1
	100*		3·0*	
11		117		4·4
		92*		3·4*
Untreated control		1		0·01

Each figure corresponds to eight plates with 8×10^5 conidia per plate.
* See text.

that the revertants were induced by the treatment, and, secondly, that the yield of revertants is independent of the time-lapse between irradiation and overlayering with LC. Even plates treated at 9 hours and overlayered immediately, or those treated and overlayered at 11 hours, show as many mutants as those irradiated earlier. The parallel behaviour of the series overlayered at 9 and 11 hours provides a check on the possible effects of changes in the u.v. sensitivity of the conidia (Reissig, 1960).

Most plates were treated according to the standard procedure: 3 ml. of minimal medium were added on top of the conidia immediately after irradiation. The values indicated with an asterisk are derived from plates in which the LC layer was added before the 3-ml. minimal layer, thus insuring immediate contact between the conidia

and the inhibitor. Slightly lower counts are given by these plates, but the difference is to small to warrant speculation.

The above results provide evidence against the occurrence of delayed mutation, delayed phenotypic expression, or selection delay in the present system.

II. *Dose dependence*

Fig. 3 shows the relation between mutation frequency and dose. The lower curve describes the total number of revertants per treated conidium. There is a net increase in the number of revertants at intermediate doses. The upper curve (mutation

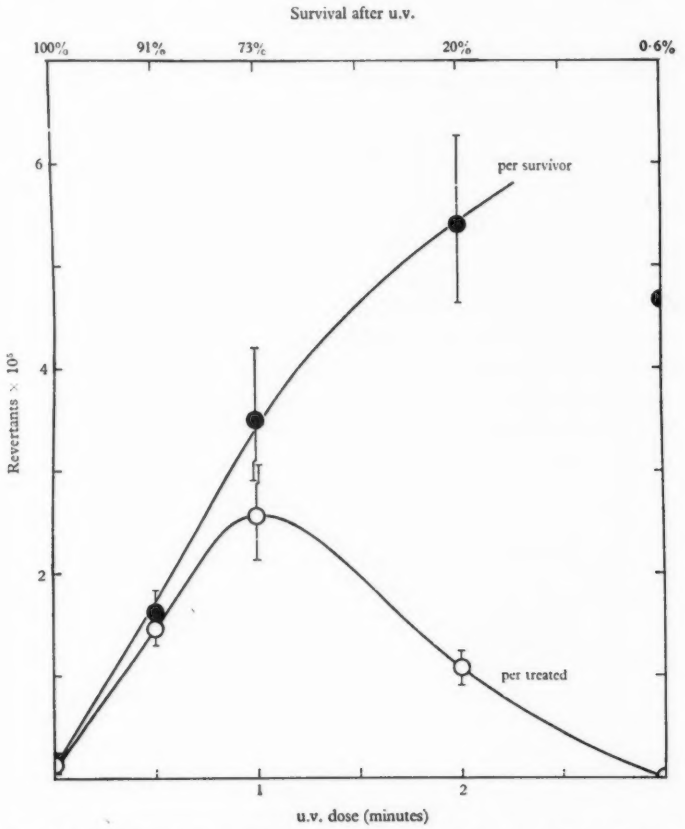

Fig. 3. Relation between reversion frequency and u.v. dose. The irradiation was performed in water. Each point represents six to twelve plates with 10^6 or 2×10^6 total spores per plate. LC was added 11 hours after plating. Confidence limits at the 95% level are indicated; except for the 3-minute points, which are based on only five colonies. The energy of the incident u.v. was $4{\cdot}5 \times 10^6$ ergs/cm²/min., as determined with the dosimeter of Latarjet. Open circles, reversion frequency per treated conidium; closed circles, reversion frequency per conidium surviving the u.v. treatment. (Treated conidium means conidium viable prior to irradiation.)

frequency per conidium surviving the u.v. treatment) appears to rise less steeply at the higher than at the lower doses. This is only weakly suggested by the points in Fig. 3. Additional data from the same experiment, which are not included in the figure, provide supporting evidence for this, and an explanation. The experiment included two parallel series; in one series LC was added at 8 hours after plating, in the other (Fig. 3) at 11 hours. The 8-hour counts were lower in every case, indicating that the physiological lag extended beyond this time. The ratios of the 11-hour to the 8-hour mutation frequencies are 1·9:1, 2·7:1, 3·3:1, and 7·2:1, at 0, $\frac{1}{2}$, 1 and 2 minutes of irradiation, respectively (actual figures were 21:11, 235:88, 117:36 and 172:24). The ratio for the 2-minute irradiation series is significantly larger than the others. This indicates that heavy doses of u.v. lengthen the physiological lag, and thus can be expected to cause a decline in the recovery of revertants.

III. *Genetics of the revertants*

(*a*) *Origin and purification of the revertants.* Thirty-three revertants were analysed genetically. One (D1) was of spontaneous origin; the rest were obtained after u.v. irradiation. Calculation from the data in Table 3 (Stevens, 1942) indicates that at most two of the mutants isolated following u.v. treatment may be of spontaneous origin ($P < 0{\cdot}05$). Thus, most or all of the mutants tested were induced by the irradiation, and therefore originated from independent events.

The thirty-three revertants were crossed to an *arg* tester, and the resultant ascospores plated on minimal and on arginine-supplemented medium. Twenty-nine of the crosses yielded prototrophic colonies; one prototroph was isolated from each cross and labelled as indicated in Table 2. In this way, the revertants were isolated as homokaryons, free of unmutated *arg* nuclei. All further work was done with these isolates.

Table 2. *Derivation of the revertants obtained after u.v. treatment*

Experiment	Survival after irradiation (percentage of untreated)	Number of revertants in comparable series		Revertants isolated
		Untreated	Irradiated	
A	78	0	38	A1 to A17
B	29	0	17	B1 to B11
C	10	0	100	C1 to C4

The prototrophs could not have originated by back mutation of the tester, since the majority of the prototrophic segregants from each cross carried the *co* marker, which was present in the treated chromosome IV but not in the tester chromosome.

Of the remaining four crosses, two yielded too few ascospores for testing. The other two (involving B9 and A15) will be discussed in section III (*f*).

(*b*) *Linkage of the reversion site to* co. The presence of the *co* marker at about 0·4 unit from *arg* provides a simple method for testing the hypothesis that reversion is due to back mutation at the *arg* locus. Twenty-five revertants and two control

arg$^+$ strains, all marked with *co*, were crossed to *co*$^+$ *arg* testers. Three different tester strains were used; the pooled data from crosses to all three testers are presented in Table 3.

Table 3. *Recombination frequencies between* co *and the reversion site*

Crosses: *co reversion* × *co*$^+$ *arg*
or *co arg*$^+$ × *co*$^+$ *arg* (control)

Group	*co* parent	Colonies on minimal (total)	Colonies on minimal (%*co*$^+$)	Colonies on arginine (%*co*$^+$)	% germination
	Control	4026	0·2	47 ± 1	84 ± 1
	Revertants:				
1	A1	1653	0·4	44 ± 4	87 ± 2
	A2	734	0·5	23 ± 3	71 ± 3
	A3	529	0·2	28 ± 3	73 ± 3
	A4	848	0·2	50 ± 3	79 ± 3
	A5	1208	0·2	52 ± 3	90 ± 2
	A6	719	0·0	47 ± 3	61 ± 3
	A7	526	0·4	47 ± 3	57 ± 5
	A8	1135	0·2	54 ± 3	84 ± 3
	A9	504	1·6	31 ± 4	70 ± 3
	A10	409	0·7	45 ± 4	70 ± 3
	A11	620	0·5	64 ± 3	61 ± 3
	B1	753	0·8	46 ± 3	92 ± 2
	B2	463	0·0	52 ± 3	95 ± 2
	B3	1502	0·3	45 ± 2	90 ± 2
	B4	1079	0·9	28 ± 3	82 ± 3
	B5	362	0·8	46 ± 4	77 ± 3
	B6	620	0·3	52 ± 3	83 ± 2
	B7	1053	0·1	50 ± 3	82 ± 3
	C1	46	0·0	33 ± 7	
	C4	14	0·0	43 ± 9	78 ± 4
2	B8	2263	0·7	44 ± 3	80 ± 4
	C2	1167	1·2	23 ± 2	73 ± 3
3	A12	751	4·7	50 ± 4	93 ± 2
	C3	1735	6·3	50 ± 2	66 ± 2
	D1	3163	1·5	48 ± 3	93 ± 2

Standard deviations are indicated for the last two columns.
The classification of the revertants in groups 2 and 3 derives from Table 4. All other strains are classed under group 1.

From the low percentage of *co*$^+$ colonies found on the minimal plates, it can be concluded that the site of reversion is closely linked with *co*; the data, however, are not homogeneous. This is perhaps not surprising since it is known that the genetic background of the strains involved can modify map distances (Stadler, 1956). The revertants used here had all been outcrossed. Alternatively, true differences in the location of the reversion site may be involved, though this point cannot be settled from the data reported so far. Incidentally, comparison of the data from crosses to the different testers showed that the heterogeneity did not derive from the use of several testers.

No explanation can be offered for the shortage of *co*$^+$ colonies on the arginine plates of some of the crosses (Table 3). This shortage affects the tester chromosome and occurs in crosses involving any one of the three testers.

(*c*) *Mapping the reversions between* co *and* pyr. By the use of three-point crosses it should be possible to eliminate the variability in map distances due to factors located outside the interval covered by the three markers. The markers used were *co*, *arg* and *pyr*. In a control series, ascospores from the cross *co arg*$^+$ *pyr*$^+$ × *co*$^+$ *arg pyr* were plated on arginine-supplemented plates. Less than 3% of the colonies were *co*$^+$, i.e. crossovers between *co*$^+$ and *pyr*$^+$. As expected, spot-tests showed these colonies to be either arginine-dependent (crossovers in the *arg-pyr* region) or prototrophic (crossovers in the *co-arg* region). The results of this cross, and of similar tests involving thirteen different revertants, are shown in Table 4.

Table 4. *Location of the reversion site in relation to* co *and* pyr

Crosses: *co reversion pyr*$^+$ × *co*$^+$ *arg pyr*
or *co arg*$^+$ *pyr*$^+$ × *co*$^+$ *arg pyr* (control)

Group	*co* parent	Colonies on arginine (total)	Colonies on arginine (%*co*$^+$)	Colonies on hydr. RNA (%*co*$^+$)	% germination	*co*$^+$ *pyr*$^+$ recombinants tested for arginine dependence: Dependent	Independent	Ratio of distances *co-reversion* / *co-pyr*
	Controls	3449	2·6	50 ± 3	84	74	7	0·09
	Revertants:							
1	A6	1618	1·3	48 ± 3	96	19	0	0·0
	A13	417	6·0	76 ± 5	57	24	0	0·0
	A14	1596	3·6	49 ± 4	90	36	6	0·14
	B1	1802	4·7	*ca.* 50	93	41	4	0·09
	B2	2622	5·3	*ca.* 50	91	50	1	0·02
	B3	1720	2·3	50 ± 3	92	33	5	0·13
	B4	2383	3·2	46 ± 3	94	39	5	0·11
	B5	1268	3·2	51 ± 4	91	31	4	0·11
2	B8	1088	4·6	25 ± 5	92	31	14	0·31
	C2	2036	1·7	32 ± 5		9	22	0·71
3	A12	2055	4·7	52 ± 2	89	0	58	1·0
	C3	2310	5·1	51 ± 4	93	0	66	1·0
	D1	3614	1·3	29 ± 3	79	0	37	1·0

Some standard deviations are indicated. For the germination percentages, the standard deviations range from 1% to 4%.

Because of the suppressor effect of *pyr* on the arginine requirement, it is not necessary to add arginine for the growth of the double mutant. The expectation for *co*$^+$ of hydrolysed RNA is slightly lower than 50% since the *arg pyr*$^+$ recombinants cannot grow on this medium. We can offer no explanation for the much larger shortage of *co*$^+$ in some of the crosses. This shortage, like the shortage of *co*$^+$ observed in some of the crosses in Table 3, affects the tester chromosome.

Testing for the *arg* phenotype in order to classify the recombinants according to genotype (Table 4) would be misleading if pseudo-wild types contributed significantly to the number of presumed recombinants. This possibility was ruled out by

crossing most of the arginine-independent recombinants to an appropriate tester, and analysing the progeny of such crosses.

The data of Table 4 have been classified in three groups. The revertants giving ratios in agreement with the expectation that the reversion site is allelic with *arg*, have been placed in group 1. Revertants giving ratios significantly different from the controls, but lower than 1·0, were classed in group 2; while those with a ratio of 1·0 were placed in group 3.

The abnormal linkage relations found in groups 2 and 3 could be due to alterations in the actual location of the *arg* locus which resulted from the mutagenic treatment (e.g. a small inversion), or to other factors affecting recombination frequencies differentially in the intervals under consideration. Alternatively, a suppressor locus might be involved. This hypothesis can be submitted to a critical test, since it postulates the persistence of the *arg* allele in the arginine-independent revertants.

(*d*) *The suppressor hypothesis.* According to this hypothesis, a suppressor closely linked or allelic to *pyr* is the cause of reversion in the case of group 3 revertants. In group 2 revertants the suppressor would be located between *arg* and *pyr*. In crosses between a suppressed revertant and a wild-type strain, *arg* would segregate out in half of the recombinants between *arg* and the suppressor. The replica plating method was used to test for the presence of *arg* in such crosses (Table 5).

Column IV of Table 5 indicates the recovery ratio in the reconstruction series of each replica-plating experiment. This ratio is expressed in terms of the actual number of colonies observed. Correction for recovery was made by multiplying the total number of *arg*$^+$ colonies by the recovery ratio (column V).

The data of column V show that *arg* is indeed still present in the group 3 revertants, but not in the others. These results can better be discussed by reference to Table 6, in which the data from Tables 3, 4 and 5 are recalculated for the same interval. A small or zero value (depending on the method of calculation) for the *arg-reversion* distance indicates that both sites are allelic, i.e. that back mutation is involved. This is the case for group 1 revertants. A value significantly larger than zero indicates non-allelism, i.e. that a suppressor is involved. This is clearly the case for group 3 mutants, where there is also good agreement for the values obtained by different methods. Conflicting results are obtained for group 2 revertants: the maximum *arg-reversion* distance calculated from Table 5 (see Table 6) is 0·3, but other methods of calculation give significantly larger distances. This discrepancy points to some kind of anomalous linkage relation in the region under consideration for group 2 revertants. Once abnormal linkage relations have to be postulated to explain the discrepancy, the differences between group 1 and group 2 revertants can also be understood. In the absence of positive evidence for the persistence of the *arg* allele in the latter strains, it appears probable that group 2 revertants are the result of back mutation at *arg*.

One might at this stage consider whether contamination, mutation or genotypes mimicking the *arg* phenotype might be responsible for the appearance of arginine-dependent segregants in some of the crosses described above. As a check against mimic genotypes, nine of the arginine-dependent segregants listed in Table 5 (six

Table 5. *Recovery of* arg *from some of the revertants by means of replica plating*

Crosses: revertant (or arg^+ control) × arg^+

Group	Strain	arg^+ segregants: arg^+	Recovery ratio	Corrected arg^+ III × IV	*arg* segregants	Distance *arg-reversion* 200 × VI / V + VI
I	II	III	IV	V	VI	VII
1	Controls	282	13/14			
		570	65/3 × 20			
		210	11/16			
	Sum			1023	0	0
	A14	1305	74/200	483	0	0
	C4	387	6/19			
		623	65/3 × 20			
	Sum			797	0	0
2	B8	1516	74/200			
		2860	71/135			
	Sum			2065	0	0
	C2	393	13/14			
		3597	71/135			
	Sum			2257	0	0
3	A12*	*ca.* 200			2	*ca.* 2
	C3-1	814	6/19		6	
	C3-2	667	74/200		5	
	C3-3	704	74/200		8	
	Sum			764	19	4·9
	D1-1	678	6/19		3	
		656	11/16		5	
	D1-2	881	11/16		3	
	Sum			1271	11	1·7

*Crosses involving A12 cannot be easily analysed by means of replica-plating in view of the partial arginine requirement of this strain (cf. section III (*g*)). The tabulated data were obtained by microscopic scanning of a plate and isolation of presumptive arginine-dependent segregants.

derived from D1 and three from C3) were crossed to an *arg* tester. Among more than 200 ascospores plated out from each cross, all were arginine-dependent, confirming that their genetic constitution was *arg*.

There are three lines of evidence supporting the recombinational origin of the *arg* segregants obtained from crosses with group 3 revertants:

(i) Various sexual isolates of C3 and D1 (C3-1, C3-2 and C3-3; D1-1 and D1-2, in Table 5) gave comparable yields of *arg* segregants.

(ii) In the cross C3-1 × wild, where the fourth chromosomes of both strains were differentially marked with *co* and co^+, all six *arg* segregants carried the marker of C3-1.

(iii) The obvious controls to rule out mutation would have been selfings of the

Table 6. *Distances between* arg *and the reversion sites calculated by different methods*

Group	Revertant	*arg-reversion* distances		
		From Table 3	From Table 4	From Table 5
1	Pooling data from all	0·1	0	0 (<0·5)
2	B8	0·4	1·2	0 (<0·3)
	C2	0·9	1·0	0 (<0·3)
3	A12	4·4	4·4	*ca.* 2
	C3	6·1	4·9	4·9
	D1	1·2	1·0	1·7

Distances estimated from Table 3 were calculated by subtracting the control *co-arg* distance from the *co-reversion* distances. The same was done for the values from Table 4 after obtaining the *co-reversion* distance from $co\text{-}pyr \times \frac{co\text{-}reversion}{co\text{-}pyr}$. The upper limits, at the 95% level of probability, are indicated for the zero values in the last column.

revertant chromosomes, i.e. crosses of C3-1 × C3-2, D1-1 × D1-2, etc. Such selfings were attempted, but turned out to be sterile or semi-sterile. Alternatively, crosses of the revertants to an *arg pyr* tester can be used as controls. As shown in Fig. 4 (A and B), arginine-dependent segregants are expected in crosses of arg^+ to such a tester, but not in crosses with suppressed mutants if the suppressor is allelic with *pyr*. The results obtained (Table 7) bear out these expectations.

(*e*) *Allelism between* pyr *and the suppressor*. The data in the last column of Table 4, and the agreement between the values in Table 6, support the hypothesis that the suppressor encountered in group 3 revertants is allelic with *pyr*. But the best evidence comes from Table 7. As illustrated in Fig. 4C, if *su* and *pyr* were not allelic, arginine-dependent segregants would be expected in crosses of the suppressed revertants to *arg pyr*. The absence of such segregants among the tested progeny (Table 7) places an upper limit of 0·1 unit on the distance between *pyr* and the suppressor (pooling the data from C3 and D1, for $P < 0{\cdot}05$).

(*f*) *Revertants yielding pyrimidine-dependent segregants*. Ascospores from the cross *co revertant B9* × co^+ *arg* were plated on minimal, on minimal plus arginine, and on minimal plus hydrolysed RNA. Only the last two media supported the growth of colonies. In the RNA plates, thirty of the colonies were *co* and two co^+. One of the latter colonies was subcultured and spot-tested. It had an absolute requirement for either hydrolysed RNA or uridine. On this basis, and with the background of previous studies on this system (Reissig, 1959), it appears that B9 resulted from forward mutation at the *pyr* locus (I in Fig. 1). The growth of the original B9 on minimal, contrasted with its inability to yield prototrophic segregants, is not unexpected from the phenotype of the heterokaryon depicted in Fig. 1. The *pyr* nucleus can complement with the other unmutated nuclei in the macroconidium, but this balance is destroyed when the different genotypes segregate after crossing.

Another revertant (A15) which failed to segregate prototrophic colonies when

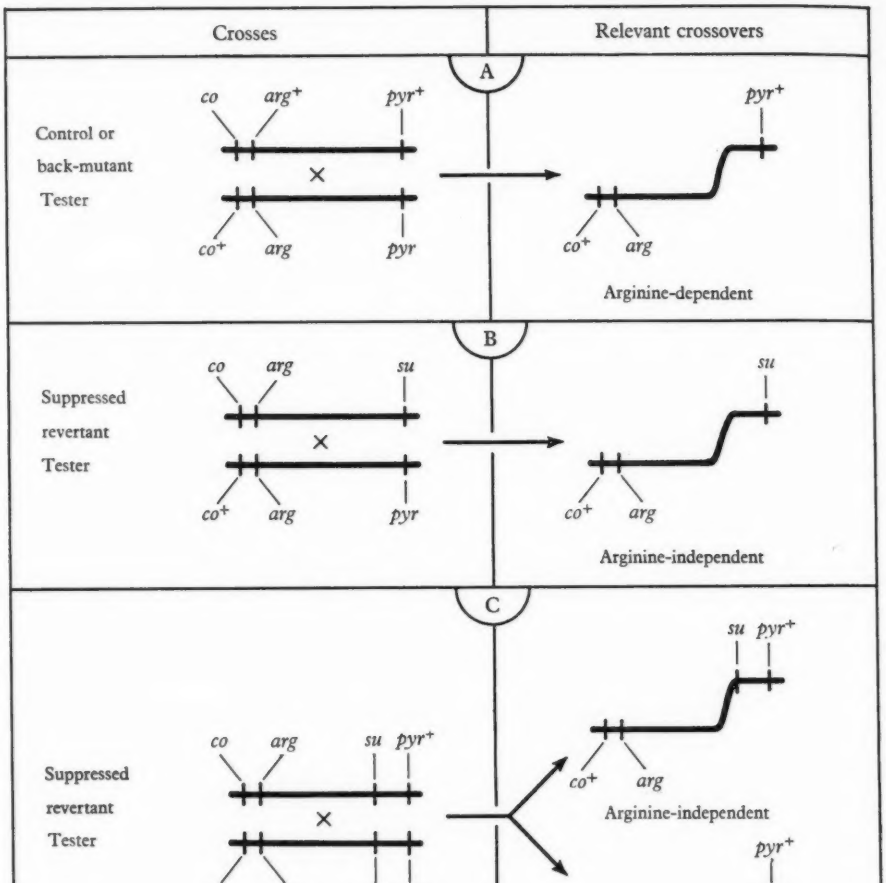

Fig. 4. Some of the segregants expected in crosses between the revertants and an *arg pyr* tester. The expectations vary with the different hypotheses concerning the genotype of the revertants: (A) Reversion is due to back mutation; arginine-dependent segregants are obtained. (B) Reversion is due to a suppressor allelic to *pyr*; no arginine-dependent segregants are obtained. (C) Reversion is due to a suppressor in the *arg-pyr* region; the frequency of arginine-dependent segregants is a function of the distance between *pyr* and the suppressor. The expectations for a suppressor located somewhere else in the genome have not been drawn, but it can readily be seen that the conclusion reached in (C) also holds for other locations of the suppressor. The actual results are shown in Table 7.

Table 7. *Testing the suppressor hypothesis in crosses to a* co$^+$ arg pyr *tester: recovery of* arg *by means of replica-plating.*

(The crosses are described in Fig. 4)

	arg$^+$ segregants				
Strains	*arg*$^+$	Recovery ratio	Corrected *arg*$^+$ II × III	*arg* segregants	Distance *reversion-pyr* $\frac{200 \times V}{IV + V}$
I	II	III	IV	V	VI
Control	459*	37/47	722	11	
	246†	37/47	194	4	
Sum			916	15‡	3·2
C3-1	1031*	28/81	713		
	1275†	28/81	441		
Sum			1154	0	0
D1-1	1200*	37/47	1889		
	924†	37/47	727		
Sum			2616	0	0

*Replica from minimal to arginine-supplemented plates. To correct for the unscored *pyr* population, the values were multiplied by 2 in column IV.

†Replica from minimal plus hydrolysed RNA plates to same plus arginine.

‡All *co*$^+$.

crossed to *arg*, was lost before its F_1 could be tested on pyrimidine-supplemented medium.

(*g*) *Partial arginine requirements among the revertants.* One might wonder whether reversion restores the capacity to grow at wild-type rate, or whether a partial requirement for arginine remains. Quantitative studies on this point were handicapped by the kind of strains used. Because of their macroconidial character, the revertants had to be purified by outcrossing. Furthermore, the *co* phenotype interfered with the measurement of growth. It may, however, be worth mentioning some preliminary observations.

Only A12 was significantly stimulated by arginine. In crosses of *co revertant A12* × *co*$^+$ *arg*$^+$, the arginine-limited growth habit segregates as if it were closely linked to *co*, indicating that this partial arginine requirement is the property of the *arg-suppressor A12* system, and not an effect of modifying genes. No arginine stimulation was detected when ascospores from crosses of C3 or D1 × *arg*$^+$ were plated, confirming that there is an intrinsic difference between the latter revertants and A12.

Hydrolysed RNA failed to stimulate any of the group 3 revertants.

4. DISCUSSION

The reported observations concerning mutational delay and delayed phenotypic expression (section 3—I) indicate *prima facie* that these two effects either do not occur, or that their occurrence takes minutes instead of hours. However, negative

evidence of this kind can seldom be conclusive (see, for instance, Lederberg, 1957). It is quite possible that the inhibitor, canavanine in this case, fails to interfere with the pathways leading to mutation and expression, even though it effectively stops the growth of sensitive cells and the germination of all conidia.

Genetic analysis of the revertants showed that those classed in group 3 became arginine-independent as the result of mutation at a suppressor locus (section 3—III (*d*)). No pyrimidine requirement is evident in these mutants (section 3—III (*g*)), and yet the suppressor seems to be allelic to *pyr*. This conclusion is based on the recombination data, which place the suppressor within 0·1 unit of *pyr* (section 3—III (*e*)), and on the functional similarity between *pyr* and the suppressor ($pyr^{su\text{-}arg}$)*. The *pyr* allele is active as a suppressor of *arg*, while the pyr^+ allele is active in the synthesis of pyrimidines. Both these activities seem to be combined in the $pyr^{su\text{-}arg}$ allele, just as they can be combined by making a heterokaryon between *arg pyr* and *arg* pyr^+. This heterokaryon can grow on minimal medium. Differences in the ability of $pyr^{su\text{-}arg}$ alleles to suppress *arg* (section 3—III (*g*)) may reflect differences in the ratios of *pyr* to pyr^+ activities.

The existence of the $pyr^{su\text{-}arg}$ allele has some bearing on the problem of heterosis. The superior growth ability of the *arg pyr*/*arg* pyr^+ heterokaryon as compared with the component homokaryons, provides an example of single-gene heterosis (Reissig, 1958). Is this a quantitative effect, or is the superiority of the heterokaryon due to the presence of alleles making qualitatively different products? Emerson (1952) has given a convincing quantitative explanation of similar cases in Neurospora. Crow (1952) suggested a criterion for distinguishing between the quantitative and the qualitative interpretations: only in the former case could intermediate alleles, capable of replacing the heterotic pair, appear. Thus, the occurrence of $pyr^{su\text{-}arg}$ lends support to the quantitative hypothesis for the pyr^+/*pyr* case. This being so, the heterosis of such pairs as pyr^+/*pyr* could only be of transient importance in evolution. Intermediate alleles like $pyr^{su\text{-}arg}$ would arise and yield a more stable system (Crow, 1952).

Little is known about the biochemical basis of the defects under consideration. Nutritional evidence (Srb & Horowitz, 1944; Srb, 1949) suggests that the *arg* mutant is blocked between ornithine and citrulline. Yet the activity of the enzyme catalysing this step (ornithine transcarbamylase) is at least as high in extracts of *arg* as in wild-type extracts†, suggesting either that the enzyme is inactive under *in vivo* conditions, or that the block affects the synthesis of carbamyl phosphate. The *pyr* alleles studied by Mitchell (1953) respond to orotic acid or to pyrimidines (see also Munkres, Woodward & Suyama, 1958). The same is true for our *pyr* isolate, except that the response to orotic acid is rather poor even in nitrate medium (Reissig & Goldberg, unpublished).

* The full symbol is $pyr3^{su\text{-}arg2}$.

† J. R. S. Fincham (personal communication), R. L. Metzenberg (personal communication) and Reissig (unpublished), working independently of each other. Fincham and Metzenberg also tested other allelic and non-allelic mutants presumed to be blocked at the same step. All had the enzyme.

The biosyntheses of arginine and pyrimidines are known to be related via carbamyl phosphate, which acts as a common precursor (Reichard, 1959). Tracer data (Heinrich, Dewey & Kidder, 1954) supports this scheme for Neurospora. This biochemical relation may underlie the observed interaction between *arg* and *pyr*. On the other hand, there is no reason to suppose that the close proximity between the *arg* and *pyr* genes plays any role in their interaction. Suppressor effects between other unlinked mutant genes affecting arginine and pyrimidine metabolism are widespread in Neurospora (Mitchell & Mitchell, 1952).

The abnormal linkage relations exhibited by group 2 revertants (section 3—III (*d*)) might be due to associated chromosomal rearrangements. De Serres (1958), studying reversions at an adenine locus, also found abnormal linkage relations among them. However, any attempt at a critical evaluation of our data would have to account also for the fact that the revertants in group 2 gave abnormal co^+/co ratios on the RNA-supplemented medium (Table 4).

SUMMARY

A system for the screening of reversions in an arginine-dependent strain of Neurospora has been developed.

Reversion resulted from any one of three events:

(*a*) Back mutation of the original *arg* gene.

(*b*) Forward mutation at the *pyr* locus. This has the pleiotropic effect of suppressing *arg* while determining a pyrimidine requirement. Interaction with the unmutated nuclei of the same conidium permits growth on minimal medium.

(*c*) Forward mutation at the *pyr* locus to alleles of intermediate activity. These alleles suppress *arg*, but do not create a pyrimidine requirement.

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Maternal effects on the rate of egg development in *Drosophila subobscura*

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1. INTRODUCTION

It was shown by Maynard Smith & Maynard Smith (1954) that outbred *Drosophila subobscura* develop more rapidly than inbred ones, as judged by the time from egg-laying to the emergence of adults, and that they are less variable at a given temperature. It was possible in these experiments to eliminate maternal and environmental effects by comparing two classes of offspring, respectively homozygous and heterozygous for genes on chromosome 5, of the same parents, and to show that more rapid development can be caused by genetic heterozygosity.

The purpose of the present investigation was to discover whether there are genetically determined differences between the rates of development of eggs, as judged by the time from laying to the hatching of larvae, and, if there are such differences, whether they depend on the genotype of the eggs themselves, or of the females which lay the eggs.

2. METHODS

Eggs from four inbred lines, *NFS*, *K*, *B* and *M*, were studied, and also F_1 eggs, and eggs obtained by crossing F_1 hybrids to unrelated inbred lines and to unrelated F_1 hybrids. The *NFS*, *K* and *B* lines are structurally homozygous for all chromosomes, and had been brother-sister mated for 50, 64 and 65 generations respectively. The *M* line had been brother-sister mated for 25 generations, but was not structurally homozygous. F_1 hybrids are written, for example, *M*/*NFS*, the female parent being written first.

The following kinds of paired mating were set up, as indicated by crosses:

	Male					
Female	*NFS*	*K*	*B*	*M*	*B*/*K*	*M*/*NFS*
NFS	×			×	×	
K		×	×			×
B		×	×			×
M	×			×	×	
B/*K*	×			×		×
M/*NFS*		×	×		×	

Forty to fifty pairs each were set up of matings involving inbred females, and fifteen to twenty pairs each of matings involving outbred females. Eggs were col-

lected from these females, 7 to 11 days after emergence, on a dark medium consisting of agar and molasses with living yeast suspension added, the medium being poured onto slips of balsa wood which fitted tightly into 3-in. by 1-in. dia. vials.

It was desired to know within one hour the time at which the eggs had been laid, and also, in order to eliminate differences in hatching time due to small temperature fluctuations, to collect eggs from all matings simultaneously, as far as this was possible. Accordingly the males were discarded after an adequate period had been allowed for mating, and eggs collected from the females for eight successive hourly periods, at the end of which time a sufficient number of eggs had been obtained from most types of mating. Unfortunately, the *K* females laid relatively few eggs, as did the *B* females mated to *M*/*NFS* males.

A complication in the procedure was imposed by the necessity of avoiding 'stored' eggs. A female can retain one fertilized egg in the vagina for many hours before laying it; such eggs hatch in a shorter time than do unstored eggs. Therefore, at the start of the experiment all the females were transferred individually onto dark medium, which was then examined at approximately hourly intervals. No female was used for the experiment proper until she had laid at least one egg. As soon as an egg was seen, the female was grouped together with a few others of the same kind, and transferred to fresh medium at hourly intervals. Only the eggs laid on these later drops of medium were recorded.

In this way some hundreds of vials were obtained containing medium on which eggs of known genotypes had been laid during known periods of one hour. Since it was desired to examine the eggs hourly for hatching, the number of vials to be examined had to be reduced. Therefore all eggs from the same kind of mating laid during the same hour were transferred to a single drop of medium, and arranged in rows to facilitate counting. Since there were still too many vials, forty-five such 'batches' of eggs were selected for actual recording of hatching time, the selection being made to give as far as possible a reasonable sample from all types of mating.

These forty-five batches of eggs were examined hourly, and the number of unhatched eggs recorded. Counting was started 36 hours after the first eggs were laid, and continued until 59 hours after the last egg was laid, at which time, since no further eggs had hatched during the last hour, it was judged safe to stop. A check of the unhatched eggs 24 hours later showed that four further eggs had hatched; these have been ignored below, but had they been included it would not have altered any of the conclusions drawn.

The vials containing eggs, except when being examined, were kept covered with a damp cloth, beneath which the temperature was between 16·5° and 17°C.

3. RESULTS

Table 1 gives for each type of mating the number of eggs hatching as a fraction of the total, and the mean hatching time in hours. The last two columns of the table give the mean and standard deviation of the hatching time for all the eggs laid by the six kinds of female.

An analysis of the results is given in Table 2, which shows that the major part of

the variation in hatching time was due to differences in the development rate of eggs laid by different kinds of female. The slowest eggs were laid by *NFS* females; this inbred line was derived by Hollingsworth & Maynard Smith (1955) from a wild-caught female by combining inbreeding with selection for slow development.

Table 1. *Fraction of eggs hatching, and mean hatching time in hours, for various types of mating*

	Male						All males	
Female	*NFS*	*K*	*B*	*M*	*B/K*	*M/NFS*	Mean	Standard deviation
NFS	30/57			33/63	27/29			
	54·37			50·39	50·93		51·88	1·69
K		16/33	5/9			7/10		
		51·19	49·00			49·71	50·43	1·52
B		39/55	28/33			6/7		
		50·00	49·10			50·83	49·59	1·25
M	43/59			26/56	43/50			
	46·61			47·42	48·21		47·41	1·28
B/K	33/33			17/36		55/56		
	48·06			46·76		48·07	47·86	0·94
M/NFS		34/46	27/30		68/68			
		47·59	46·52		46·03		46·54	1·13

Although selection was relaxed after seven generations, *NFS* flies still take 3 to 4 days longer to emerge than flies from other inbred lines. Eggs laid by *B* and *K* females were intermediate in development rate, and the fastest eggs were laid by the F_1 hybrid females *B/K* and *M/NFS*, and by inbred *M* females.

The effects on hatching time of the zygotic nucleus can be estimated by comparing eggs laid by females of a given type mated to different kinds of males. These effects are much smaller than the maternal effects. Before deciding whether they are statistically significant, an estimate of the 'error variance' must be made.

Table 2. *Analysis of variance of egg hatching time*

	Sum of squares	Degrees of freedom	Variance	*P*
Within batches	794·1	492	1·614	
Between batches of the same genotype	120·2	27	4·45	< 0·001*
Total within genotypes	914·3	519		
Between males { with *NFS* females	283·0	2	141·5	< 0·001†
Between males { with other females	178·9	10	17·9	< 0·01†
Total within females	1376·2	531		
Between females	1888·0	5	377·6	
Total	3264·2	536		

* Comparison of variances within and between batches.
† Comparison with the between-batch variance.

The first two rows of Table 2 compare the within-batch variance, due to differences between eggs laid by females from the same type of mating in the same hour, with the between-batch variance, due to differences between eggs laid by females from

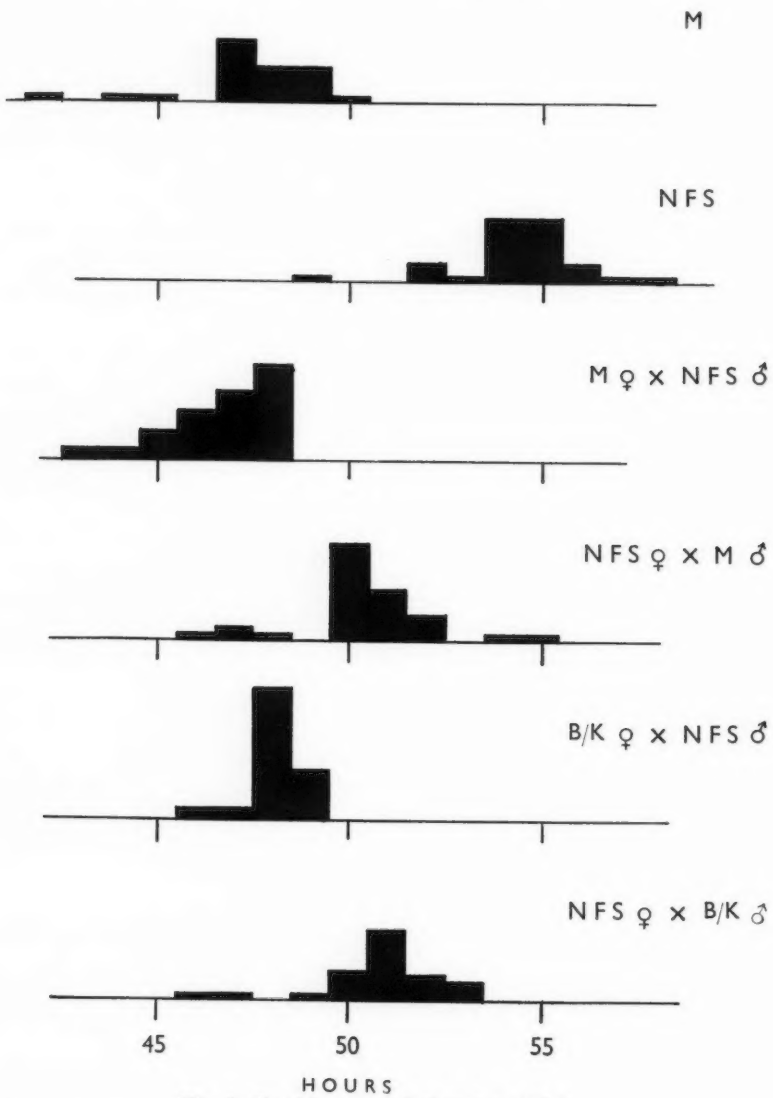

Fig. 1. Hatching times in hours at 17° C.

the same type of mating in different hourly periods. The latter variance, although it contributes little to the total, is significantly greater than the within-batch variance. Presumably this reflects slight differences in the temperature or humidity

to which different batches were exposed. Therefore, in estimating the significance of nuclear effects, these must be compared with the between-batch variance.

Differences due to the zygotic nucleus have been analysed into two parts. First, there is a highly significant contribution to the total variance due to the fact that eggs laid by *NFS* females develop at different rates according to the male which fertilizes them. Table 1 shows that *NFS* eggs fertilized by *NFS* sperm took about 4 hours longer to hatch than did *NFS* eggs fertilized by sperm from an unrelated male; it does not matter whether the unrelated male is inbred or outbred.

The remaining effects of the zygotic nucleus, for eggs laid by females other than *NFS*, are just significant at the 0·01 probability level, but it is far from clear what they signify. As Table 1 shows, it is not true that eggs laid by inbred females (other than *NFS*) develop more slowly if fertilized by sperm from the same inbred line, nor is it true that eggs develop more slowly if fertilized by *NFS* sperm.

Finally, the data give some information on the variability of development rate. The standard deviations given in the last column of Table 1 are the square roots of the within-batch variances for each kind of female; the values would have been slightly higher had the between-batch variances been included. It will be seen that eggs laid by F_1 hybrid females are less variable than the eggs laid by inbred females.

The various conclusions are illustrated in Fig. 1, a selection of the data which shows the differences between reciprocal hybrids due to the maternal effects, and also the slow development and greater variability of eggs laid by *NFS* females, particularly when fertilized by *NFS* sperm.

4. CONCLUSIONS

The rate of development of *Drosophila* eggs, from laying to hatching, is in the main determined by the female laying the eggs, and only to a small extent by the genotype of the embryo. This agrees with the finding of Moore (1933), working with species hybrids in echinoderms, that cleavage rate is determined by the cytoplasm and not by the nucleus. Later, in the larval and pupal development of *Drosophila*, the work of Maynard Smith & Maynard Smith (1954), and other unpublished data, suggest that the nuclear genotype of the individual is of major importance in determining development rate. But this may not always be the case; Clark (1957) found that there was a large difference in development rate between the species *D. setifemur* and *D. spinofemora*, and that F_1 hybrids between these species resembled their mothers in the time taken to develop from egg to adult.

One case only was found in which the male parent had a large effect on the rate of egg development. Eggs laid by inbred *NFS* females develop more rapidly if fertilized by unrelated males. In this case it seems that the sperm can in part compensate for some deficiency of the egg cytoplasm, probably through the chromosomes which it contributes to the zygotic nucleus.

Although the rate of egg development is in the main determined maternally, and of later development by the genotype of the developing individual, it is interesting that those genotypes which cause individuals to develop rapidly also cause those individuals, if female, to lay eggs which develop rapidly. Thus F_1 hybrids between

inbred lines develop into adults more rapidly, and with less variation of development time, than do inbred individuals, and F_1 hybrid females also lay eggs which develop more rapidly and are more uniform than the eggs laid by inbred females. Similarly, *NFS* individuals take longer than other inbred individuals to develop into adults, and *NFS* females lay eggs which take longer to hatch.

SUMMARY

The time in hours from laying to hatching at 17°C. has been measured for eggs from four inbred lines of *Drosophila subobscura*, and also for F_1 eggs, and for eggs obtained by crossing F_1 hybrids to unrelated inbred lines and to unrelated F_1 hybrids.

Eggs laid by females of a given genotype have a characteristic rate of development, which, with one exception, is not greatly influenced by the genotype of the male parent. F_1 hybrid females laid eggs which were more uniform in hatching time than eggs laid by inbred females, and which developed more rapidly than eggs laid by three of the four kinds of inbred female studied. The slowest-developing eggs were laid by females from the *NFS* inbred line, a line originally selected for slow development from egg to adult.

The male parent, and hence the zygotic nucleus, had an appreciable effect on the rate of egg development only for eggs laid by *NFS* females; these eggs developed more rapidly if fertilized by sperm from an unrelated male.

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The induction of dominant lethal mutations in rats and mice with triethylenemelamine (TEM)

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1. INTRODUCTION

When the work herein described was begun, it was not yet known that TEM (= tretamine = triethylenemelamine = 2:4:6 tri(ethyleneimino)-1:3:5 triazine) was a mutagen in mammals: only that it reduced the fertility of injected male rats in the first weeks after injection (Bock & Jackson, 1957). The first task was, therefore, to confirm that this infertility was in fact due to the induction of dominant lethal mutations. This has since been done in the rat by Steinberger *et al.* (1959) and in the mouse by Cattanach & Edwards (1958), who went on to show (Cattanach, 1959) that translocations were also induced. In addition to confirming this published work we have also revealed a pattern of sensitivity in the various germ-cell stages which shows both resemblances to and differences from the sensitivity pattern established for X-rays (Bateman, 1958*a*).

The infertility of the treated male rats could conceivably have been due to numerical or functional oligospermy. By functional oligospermy we mean that most of the sperm inseminated are incapable of fertilization. (Aspermy could be excluded because the criterion of mating was the presence of sperm in vaginal swabs and consequently aspermic matings would not have been recorded as matings.) Severe oligospermy, whether numerical or functional, would reduce the proportion of fertilized eggs. This would reduce the rate of implantation, but all implanted eggs would develop normally.

Dominant lethal mutations in sperm, on the other hand, do not affect their capacity for fertilization, but sooner or later kill the fertilized egg. They are generally assumed to arise from unhealed chromosome breaks, which are freely produced both by chemical mutagens and ionizing rays. As shown by an analysis of X-ray induced mutations in mice (Bateman, 1958*b*), they may express themselves either as unimplanted eggs (which are indistinguishable from unfertilized eggs when dissected after implantation) or as deciduomata. These latter consist of entirely maternal tissue which has been stimulated into growth by an egg which dies shortly afterwards. Though by definition a dominant lethal may kill the embryo carrying it at any time during gestation, in practice those produced by mutagens rarely kill established embryos.

When the mutation rate is high (as following a high dose of mutagen) pre-implantation losses are high, or even complete, simulating failure of fertilization.

For a critical test of dominant lethal induction, therefore, the optimum dose of reagent is the maximum which permits a high proportion of the fertilized eggs to be implanted.

2. ACTION OF TEM ON MALE RATS

A wide range of doses, from 0·4 mg./kg. of body-weight down to 0·025 mg./kg., was administered i.p. to male rats. Single males were placed with single females of proven fertility for weekly periods. In one series an attempt was made to increase the amount of information per male by giving each male two females per week, but it was found that the males generally could not mate effectively with more than one female per week. The doubled consumption of females barely increased the information per male, so we returned to the one female per male per week routine.

As mentioned above, matings were identified by means of daily vaginal swabs, which enabled one to follow oestrus and look for the presence of sperm. The simpler method of inspection for vaginal plugs, so effective for mice, is inapplicable, presumably because the plugs have fallen out before inspection. There is the advantage that aspermic matings, which would result in pseudopregnancy (suspension of oestrus), are excluded from the fertility studies, and the corresponding disadvantage that aspermy induced by the treatment is not distinguished from failure to inseminate. Aspermy is proved only by histological studies of epididymis and testis.

Dissections of uteri were made 14 days from mating. This corresponds to the stage reached at 12 days from mating in the mouse (Bateman, 1958*a*) with one important exception, that in pregnancies without live foetuses the deciduomata of the mouse have already been shed at 12 days though they are still in the uterine lumen, but the deciduomata of the rat at 14 days are still firmly attached to the myometrium.

Slight confusion was encountered at first in counting the rat corpora lutea, as there appeared to be two distinct sizes. It was found to be the large ones that represented the ovulations, so the smaller ones were ignored.

Table 1 summarizes all the rat data except for the controls, which are given in Table 2. The abbreviations used are the same as in a previous paper (Bateman, 1958*a*) but are redefined in the legend to the table. The ratio ED/IMP is only approximately proportional to the incidence of dominant lethal mutations (see Bateman, 1958*b*) but is suitable for our purposes. The main deficiency of ED/IMP is that it cannot exceed unity however high the mutation rate may be. It is most useful therefore at lower mutation rates: up to one mutation per egg. When the mutation rate is high this will affect the implantation rate by increasing (CL—IMP) the eggs which fail to implant. PSP will also reflect mutation rate when this is high enough to cause the death of all the eggs in a pregnancy.

It is clearly established from Table 1 that the reduced fertility of male rats for the first 4 weeks after treatment with TEM is due in part to the induction of dominant lethal mutations represented by ED/IMP, and for the rest to the less specific low IMP and high PSP at higher dose levels. This confirms the conclusions of Steinberger, Nelson, Boccabella & Dixon (1959). Furthermore, the acute sterility

Table 1. *Analysis of matings of treated male rats by dissection at* 14 *days after mating. Week number denotes the week after treatment in which mating occurred*

CL = number of corpora lutea (excluding pseudopregnancy).
IMP = number of implantations alive and dead.
ED = number of deciduomata (i.e. ova dying soon after implantation).
PSP = ratio of pseudopregnancies (no implantations of any kind) to total matings.

Dose (mg./kg.)		Week 1	2	3	4	5	6
0·4	CL	94	72	75	10	73	
	IMP	65	12	21	5	41	
	ED	54	10	21	5	22	
	PSP	1/10	3/9	3/10	5/6	0/6	
	ED/IMP	**0·83**	**0·83**	**1·00**	**1·00**	**0·54**	
0·2	CL	76	75	41	9	54	70
	IMP	64	41	22	1	55	65
	ED	43	38	21	1	15	5
	PSP	0/7	1/9	2/7	2/3	0/5	0/5
	ED/IMP	**0·67**	**0·93**	**0·95**	**1·00**	**0·27**	**0·08**
0·1	CL	21	18	29	43	33	35
	IMP	21	15	17	23	30	31
	ED	4	14	8	20	4	1
	PSP	0/2	0/2	1/4	3/7	2/5	0/3
	ED/IMP	**0·19**	**0·93**	**0·47**	**0·87**	**0·13**	**0·03**
0·05	CL	64	66	66	51	59	53
	IMP	54	64	57	39	55	52
	ED	11	20	16	28	4	4
	PSP	1/7	2/8	0/7	1/5	0/5	0/5
	ED/IMP	**0·20**	**0·31**	**0·28**	**0·72**	**0·07**	**0·08**
0·025	CL	58	52	56	59	39	
	IMP	49	50	55	53	36	
	ED	8	4	2	9	4	
	PSP	0/5	0/5	0/5	0/5	1/4	
	ED/IMP	**0·16**	**0·08**	**0·04**	**0·17**	**0·11**	

experienced in the fourth week is due to a peak sensitivity to induction of mutations in that week. That is, sperm utilized in the fourth week was in a stage hypersensitive to the mutagenic action of TEM at the time of treatment. A break-down of the first week's matings disclosed that mating in the first half of the week had a consistently lower mutation rate than later matings.

A check was made on the possibility that the PSP following high doses was due to a new phenomenon, such as impairment of the fertilizing capacity of the sperm.

Ova from matings in the fourth week after 0·4 mg./kg. (when PSP is very frequent) were examined on the second day after mating. All were fertilized and most had undergone first cleavage. But instead of a single large nucleus in each blastomere there were several nuclei of varying sizes. Evidently the fertilizing capacity of the sperm was unimpaired, the micronuclei being due to multiple breakage of the chromosomes. Hence the failure of the ova to survive to implantation. Thus, in this material, reduced IMP and high PSP were also manifestations of dominant lethal mutations.

There is an abrupt change from the fourth to the fifth week, which except at high doses was similar to the control, with full fertility and almost complete absence of dead embryos. The sixth week was like the controls even at maximum dose levels. These results are in accordance with Bock and Jackson's fertility data (1957).

It is desirable to identify the stage in spermiogenesis which is showing the hypersensitivity; in other words, which stage matures in the fourth week after treatment. There is a superficial resemblance between this hypersensitivity and that found in early spermatids of the mouse following X-rays (Bateman, 1958*a*). A more direct comparison can be made with the data of Craig, Fox & Jackson (1959) on the fertility of male rats following 300 r X-rays. There were two periods of low fertility; the fourth week and a period over weeks 9 and 10. Apart from the extended time-scale, this bears such a close resemblance to the mouse data after 200 r (Bateman, 1958*a*) that the respective sensitive stages may be presumed to be homologous and so also the intervening fertile periods. The timing of spermatogenesis in the mouse has been known for some time from the work of Oakberg (1956) and Sirlin & Edwards (1957). More recently similar studies have been made in the rat (Clermont & Leblond, 1960) and these fully confirm this interpretation. In the terminology of these workers the term 'sperm' is confined to stages outside the testis and 'spermatid' for all post-meiotic stages found in the testis however close their morphology to that of sperm. Since 'spermatid' then covers a wide range of cell types with a wide range of mutagenic sensitivity it is necessary to subdivide them in some way. I shall therefore use the terms 'late', 'mid' and 'early'. Though the classification is based upon the order in which they mature following treatment they will correspond roughly to the morphological types: nuclei resembling mature sperm, elongating nuclei, and spherical nuclei respectively.

From the above considerations it can be concluded that mid and early rat spermatids at treatment will be used for inseminations in the fourth and fifth weeks; spermatocytes in the sixth to eighth week; and spermatogonia type B and intermediate in the ninth and tenth week. It follows from this that in terms of their response to the mutagenic action of TEM, rat sperm and late spermatids are moderately sensitive, mid spermatids are hypersensitive, early spermatids are slightly sensitive and spermatocytes are highly resistant. Using higher doses and mating the rats for longer periods, Jackson (unpublished) found sterility in the ninth and tenth weeks which must have been due to sterilization of spermatogonia type B.

The results presented in Table 2, where data from the first 4 weeks' matings are pooled, suggest an unusual dose-dependence of the mutagenic effects of TEM. The

Table 2. *Analysis of pregnancies initiated by male rats injected with various doses of TEM. Symbols as for Table* 1. *Matings in the first four weeks after treatment have been pooled. The final estimate of mutation rate, $-ln(1-ED/IMP)$, is based, however, on the unweighted mean of the four weeks*

	Dose in mg./kg.					
	0·4	0·2	0·1	0·05	0·025	Control
Number of matings	35	26	15	27	20	10
CL	251	201	111	247	225	93
IMP	103	128	76	214	207	85
ED	90	103	46	75	23	4
PSP	0·34	0·19	0·27	0·15	0·00	0·00
(CL − IMP)/CL	0·59	0·36	0·32	0·13	0·08	0·09
Gross ED/IMP	0·87	0·80	0·61	0·35	0·11	0·05
Mean ED/IMP	0·915	0·888	0·615	0·378	0·113	0·047
−ln(1 − Mean ED/IMP)	**2·465**	**2·185**	**0·954**	**0·474**	**0·119**	**0·048**

effect of 0·025 mg./kg. is disproportionately low, much less than mid-way between the control level and 0·05 mg./kg. As already indicated, the ratio ED/IMP is an imperfect quantitative measure of mutation rate. It has therefore been converted to the parameter advocated by Bateman (1958*b*): namely −ln[1−(ED/IMP)], and

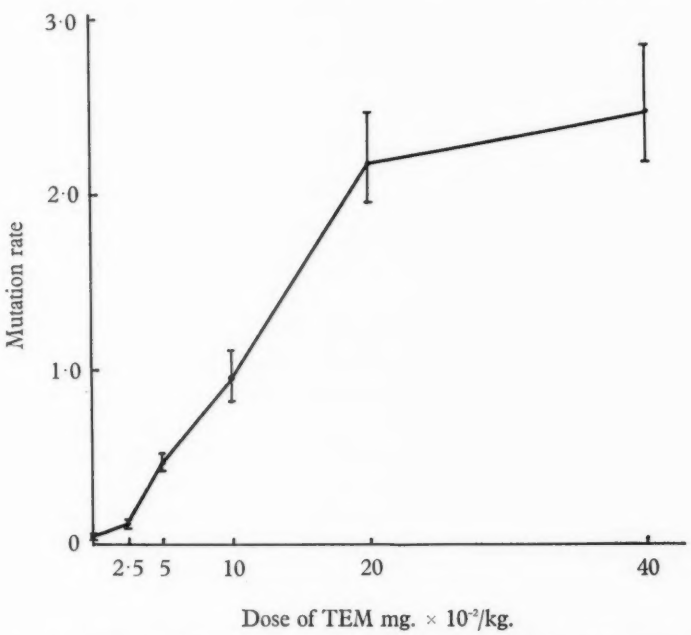

Fig. 1. Dependence of mutation rate [−ln(1−ED/IMP)] in the rat on dose of TEM. The depression at the lowest dose (0·025 mg./kg.) might indicate a threshold effect. The lines representing the standard errors are derived from those calculated for ED/IMP using the binomial $[\sqrt{(pq/k)}]$.

this has been plotted against dose in Fig. 1. It would be premature to discuss whether this signifies a threshold effect for chemical mutagens.

3. ACTION OF TEM ON MALE MICE

The pattern of sensitivity to TEM with successive sperm samples, as published by Cattanach & Edwards (1958) and Cattanach (1959), appeared to contrast strongly with that for TEM in rats as presented above, and also for X-rays in mice (Bateman, 1958*a*). It was therefore felt necessary to repeat the work in our own laboratory, with the same TEM as had been used on the rats and the same strain of mice as had been irradiated.

Owing to the freer mating of mice it was possible to obtain a day-by-day analysis of sperm instead of the week-by-week analysis obtainable with rats. Two dose levels were administered: single injections i.p. of 0·2 mg./kg. to eight mice and 0·8 mg./kg. to four mice. Each male at the high dose was given one female per week starting with the second week. The purpose of this latter was merely to time the return of fertility at a high dose level. Matings were timed by the appearance of vaginal plugs.

Table 3. *Dominant lethal mutations induced in male mice by a single injection of 0·2 mg./kg. of TEM on day 0. Symbols as in Table 1. Though data were obtained for each day separately, for the purposes of the table adjacent days have been combined so that each estimate of ED/IMP is based on a minimum of thirty IMP.*

	Week 1			Week 2				Week 3				Week 4		
Day	0–2	3	5–6	7–8	9–10	11–12	13–14	15–17	18	19	20–21	23	24–28	Control
CL	47	58	55	67	84	71	115	59	67	67	85	29	65	187
IMP	48	40	45	37	75	55	68	51	58	60	71	29	61	179
ED	8	18	18	17	35	30	36	11	7	4	5	1	8	8
ED/IMP	0·17	0·44	0·40	0·46	0·44	0·55	0·53	0·22	0·12	0·07	0·07	0·03	0·13	0·05
Week's ED/IMP		0·331			0·505				0·195			0·092		

The data presented in Table 3 confirm the results of Cattanach & Edwards. The features of the sensitivity pattern are as follows. The mutation rate rises rapidly to a steady level from the third day after treatment to the tenth. Days 11–14 show a slight further rise which is followed by a sudden drop to the fifteenth day which develops into a steady decline, reaching the control level by the twenty-second day. The contrast with the sensitivity pattern following X-rays is clearly seen in Fig. 2, where 500 r is compared with 0·2 mg./kg. of TEM. The two mutagens give the *same* mutation rate from days 3–12, but very different results before and after. The absence of values beyond the nineteenth day for X-rays is due to the onset of complete sterility in contrast to the full fertility of TEM. The high value for day 3 with X-rays, though not significant in itself, is confirmed by more extensive data at other doses (Bateman, 1958*a*).

The translation of days from treatment on which mating occurred into stage at which the germ-cells were treated is shown in Table 4. Taking the sensitivity of the

period 3 12 days as standard (late spermatids and epididymal sperm) ripe sperm and early spermatids have a high sensitivity to the induction of mutation by X-rays, and a low sensitivity to TEM. The sensitivity of mid and early spermatids to TEM is not uniform but steadily decreases with immaturity. Finally spermatocytes, completely sterilized (i.e. incapable of producing viable sperm) by the X-rays, are resistant both in terms of sterilisation and of mutation to TEM.

The resistance of spermatocytes to TEM was further tested at the 0·8 mg./kg. dose level. Though matings occurred regularly each week, no litters were obtained from second-week matings, nor from the third-week, though blood in the vagina of one

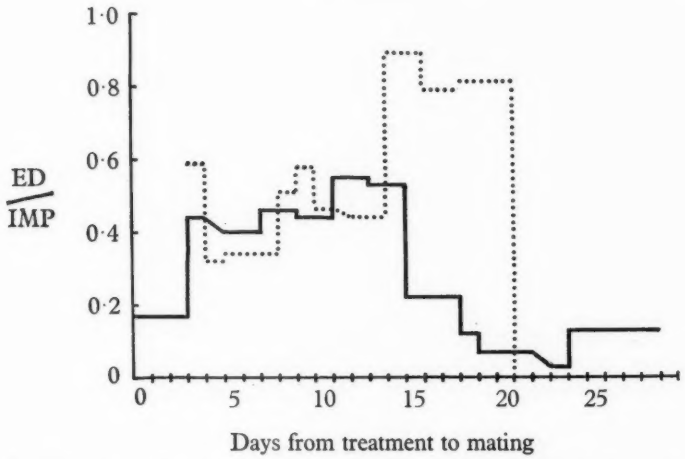

Fig. 2. Histogram showing daily variation in the ratio ED/IMP in matings of treated male mice. Entire line: 0·2 mg. TEM/kg. (present data): Dotted line: 500 r X-rays (from Bateman, 1958*a*). Where there are less than thirty implants per day, neighbouring days have been pooled to make up a total of at least thirty.

female 12 days after mating indicated pregnancy with abortion of the entire litter. Matings in the fourth week produced litter sizes of 3, 10, 13 and 11. In the fifth week the corresponding litters were 13, 17, 16 and 13. The litters conceived in the fourth week appear to be slightly subnormal in size. It can be concluded that spermatocytes are much more resistant than spermatids to TEM and early spermatocytes more resistant than late ones.

4. DISCUSSION

There are three possible comparisons to be made: between X-rays and TEM, between rats and mice, and interactions between species and mutagen. One could make this on a purely temporal basis, according to the time from treatment to mating. But a much more meaningful comparison, because of the different timing of spermatogenesis in rats and mice, would be according to the germ-cell treated. The tran-

slation of period from treatment to mating into stage treated is shown in Table 4, together with, in semi-quantitative terms, the sensitivity of each stage to mutagen in respect of the induction of dominant lethals on the one hand and in respect of the sterilization of the cell on the other. The table combines the author's data with those from several sources previously cited in the text. By 'sterilization' is meant here, the inability of the cell to form viable division products, whether of type A spermatogonia to form type B, type B to form spermatocytes, or of spermatocytes to form viable sperm. The term 'sterilization' is used in preference to 'cytotoxic' or 'cell lethal' because it covers not only death of the treated cell but also the inability of that cell to produce functional sperm. The sterilization of sperm would mean the destruction of its fertilizing ability, something which no mutagen has been found to do.

On such a definition the sensitivities of rat and mouse spermatogonia to the sterilizing effects of X-rays are similar, even though the histological results (cf. Mandl, 1959, and Oakberg, 1955) are so contrasting. Apparently, whilst rat and mouse spermatogonia are equally damaged by, say, 500 rad, the death of the cell is within a few hours in the mouse but is delayed for several days in the rat. This much is certain, that after 500 rad to the male rat (Craig *et al.*, 1959) as with the male mouse, there is complete sterility during the interval corresponding to irradiation of spermatogonia and spermatocytes.

The most imperfect of the four sets of data on which the comparisons are based is the effect of X-rays on the rat (Craig *et al.*, 1959), since it is concerned only with mean litter sizes, and cannot therefore discriminate between dominant lethals and cell sterilization. It can safely be assumed, however, that reductions in litter size for matings in the first five weeks are due to dominant lethals. After allowing for the difference in timing, the parallelism with the mouse data is so close that it is justifiable to conclude that mouse and rat germ-cells show the same sensitivity pattern to X-rays.

In making comparisons of sensitivity it is necessary to adopt a standard as the basis of comparison. I have taken as this standard the production of an ED/IMP of 0·5 in epididymal sperm. This is produced by 500-rad X-rays in rats and mice, by 0·2 mg./kg. of TEM in the mouse and 0·1 mg./kg. of TEM in the rat. These doses are only approximate, but this is good enough for our purposes as we are only concerned with major fluctuations in sensitivity from the standard. The standard dose for X-rays in the rat is based on a small-scale unpublished experiment in this laboratory, which is consistent with the fertility changes reported by Craig *et al.* (1959).

Let us first consider the comparison between X-rays and TEM, confining ourselves to features common to both species. Similarities are the immunity of post-meiotic stages to any sterilizing effect, and the absence of dominant lethals from treated spermatogonia. This latter is due to a failure to recover them in the sperm, rather than failure to induce them. That is, it lies in the nature of the mutations rather than of the mutagen. That TEM can induce chromosome breaks in type A spermatogonia has been shown by Ockey (1959), who observed aberrations in these

Table 4. *A comparison of the relative sensitivities of the various stages of spermatogenesis to the mutagenic and cell-sterilizing actions of X-rays and TEM in the mouse and rat. The dominant lethal sensitivity of spermatogonia is entered as nil, not because the mutations cannot be induced, but because they are not recoverable in the sperm*

	Stage of germ-cell treated						
	Sperm		Spermatid			Spermatogonium	
	in vas deferens	in epididymis	late	mid: early	Spermatocyte	Type B	Type A
Week after treatment in which it is used as sperm — Mouse	1	1–1	2	3	4–5	6–7	8 onwards
Week after treatment in which it is used as sperm — Rat	1	1–2	3	4–5	6–8	9–10	11 onwards
Dominant lethals							
X-rays — Mouse and Rat	High	STANDARD	Standard	High	Standard	NIL	NIL
TEM — Mouse	Low	STANDARD	Standard	Low	Almost immune	NIL	NIL
TEM — Rat	Low	STANDARD	Standard	High: Low			
Cell sterilization							
X-rays — Mouse and Rat	Immune	Immune	Immune	Immune	Moderate	Very high	Moderate
TEM — Mouse and Rat	Immune	Immune	Immune	Immune	Immune	Almost immune	Immune

spermatogonia in the field vole (*Microtus agrestis*) after TEM, though the maximum rate observed was low (1·8 per 100 cells).

One difference between mutagens is that mature sperm is more sensitive than the standard, immature sperm, to X-rays but less sensitive to TEM. A second difference is that pre-meiotic stages are sterilized by the standard X-ray dose, but there is no detectable effect of the standard TEM dose. Of the three pre-meiotic cell types listed in Table 4, type B spermatogonia are the most sensitive to both reagents. At lower doses of X-rays (e.g. 100 rad to mouse; Oakberg, 1955) and at higher doses of TEM (e.g. 1 mg./kg. to rat; Jackson, unpublished), they are the only stage to be sterilized.

A third difference only becomes apparent at lower X-ray doses than the standard. It is only at doses below the sterilizing dose for spermatocytes (e.g. 200 rad mouse) that one can expect to detect any mutagenic effect of X-rays. It is then found that spermatocytes have a sensitivity to X-rays equal to immature sperm (Bateman, 1958*a*), contrasting with their high resistance to TEM.

The next comparison is between rats and mice. Both species react similarly to X-rays but differently to TEM. There is thus no consistent difference between the species.

We come therefore to the third comparison: the interaction between species and mutagen. Both species show the same pattern of resistance to the sterilizing effect of TEM, which is almost confined to type B spermatogonia at the doses used in this work, though really high doses (five consecutive daily doses of 0·4 mg./kg. rats; Jackson, personal communication) can produce permanent sterilization. The species-mutagen interaction is confined to the yield of dominant lethals from spermatids treated with TEM. Mid-stage rat spermatids are hypersensitive whilst the earliest stages are resistant. Both mid and early mouse spermatids, on the other hand, are highly resistant.

The main consistent differences between X-rays and TEM concern the pre-meiotic stages. The simplest explanation would be one of penetrance. The penetrance of X-rays is of course uniform throughout testis and epididymis, but it might be that TEM has much readier access to post-meiotic stages in testis and epididymis than elsewhere. This would explain the parallel immunity of all pre-meiotic stages to sterilization, and of spermatocytes to mutation. The only further assumption that has to be made is that mid-stage mouse spermatids, unlike mid-stage rat spermatids, are largely sheltered from TEM. Only experiments with labelled TEM could test this hypothesis.

It is not easy to compare the sensitivity pattern in mouse and rat with that obtained in *Drosophila* by Fahmy & Fahmy (1954). This is because, in *Drosophila*, unlike rats and mice, mature sperm which is not used in matings is accumulated in the seminal vesicles. A fast mating rate is therefore necessary before one can attempt to equate interval from treatment to mating with stage of treated germ-cell being sampled. The Fahmys provided only one female per male every 3 days, and this is certain to produce accumulation of earlier maturing sperm which would be used with later maturing sperm. It is not possible, therefore, to equate successive 'broods' (3-day mating periods) with particular germ-cell stages except that the

first brood would consist of sperm which was mature at treatment, whereas the sperm in later broods would be, on average, less mature at treatment. In that case, the fall in mutation rate in *Drosophila* from the first to the second brood must mean that mature sperm is more sensitive than immature sperm, which is contrary to our mammals. No further comparison is justified.

SUMMARY

1. TEM resembles X-rays in inducing dominant lethal mutations in the sperm of rats and mice and sterilizing type B spermatogonia. Beyond this, however, there are several important differences.

2. The relative ease with which TEM and X-rays affect sperm and spermatogonia varies greatly. The X-ray dose which produces 50% dominant lethals in sperm (500 rad) sterilizes spermatocytes and type B spermatogonia, and has such a drastic effect on type A spermatogonia that recovery of fertility is delayed for $2\frac{1}{2}$ months. The TEM dose which produces the same mutation rate in sperm (0·1 mg./kg. rat) has no detectable effect on pre-meiotic stages. Even 1 mg./kg. only sterilizes the most sensitive stage, type B spermatogonia.

3. Taking immature sperm as the standard, mature sperm are more sensitive to X-rays, but less sensitive to TEM, and early spermatids, the most sensitive stage to X-ray-induced dominant lethals, are highly resistant to TEM.

4. Spermatocytes, in which X-rays yield a mutation rate equal to immature sperm, are highly resistant to TEM.

5. To produce the same mutation rate in immature sperm, mice require twice as much TEM as the rat in mg./kg., though approximately the same X-ray dose.

6. In contrast to mid-stage rat spermatids, which are the most sensitive stage to TEM, mouse mid-stage spermatids are resistant.

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The fertility of mice selected for large or small body size

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Selection for large or small body size results in considerable changes in the adult body weight of the selected lines. Examples of the effects of selection for body size are to be found among domestic livestock (Comstock, Winters, Jordan & Kiser, 1942; Krider, Fairbanks, Carroll & Roberts, 1946; Dickerson & Grimes, 1947; etc.) and experimental animals, e.g. the mouse (Goodale, 1938; MacArthur, 1944*a*, *b*, 1949; Falconer, 1953, 1955). The usefulness of selection programmes is limited if fertility is impaired, and changes in body size are often associated with changes in reproductive traits such as the time of onset of sexual maturity, litter size, etc. (e.g. Hertzer & Brier, 1940; Phillips & Zeller, 1943; MacArthur, 1944*a*, *b*, 1949). The present paper describes the effects of long-term selection for large and small body size on the reproductive physiology of two unrelated strains of mice.

MATERIAL

Two strains of mice, both selected by Dr D. S. Falconer of this Institute, were available. Strain *N* had been selected for large (line *NL*) or small (line *NS*) body size, and an unselected control line (*NC*) had been constructed at a later date than the selected lines. The origin and history of strain *N* were described by Falconer (1953, 1955). Strain *C* had also been selected for large and small body size (lines *CL* and *CS* respectively), no control line being available. The strain is described by Falconer (1960) (the lines here designated *CL* and *CS* were those selected on a normal diet, their full designation being *CFL* and *CFS*). Selection was based on body weight at 6 weeks of age in strain *N*, and on weight gain between 3 to 6 weeks of age in strain *C*.

Most of the data were derived from strain *N*. During the course of selection, the generation interval in the small line had increased due to delay in the arrival of first litters. The contemporaneous generations available for a comparative study were 25–31 in *NS*, 18–25 in *NC*, and 34–40 in *NL*. Considerable differences in body weight existed between the three lines at the time of this study (see Table 5), the response to selection being apparently complete.

Strain *C* had been selected for fewer generations, and both large and small lines were still responding to selection. The mice used came from generations 16–20 in *CL*, and 16–19 in *CS*. There was no evidence of delay in sexual maturity in line

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CS. Considerable differences in body weight also existed between *CL* and *CS* mice at the commencement of this work.

The mice used were between 6 and 25 weeks of age. All of the females were nulliparous.

THE MEASUREMENT OF FERTILITY

The interval between pairing males and females and the arrival of the subsequent litter was recorded as an overall indication of the fertility in each line, and of the interval between pairing and mating. ('Pairing' is used to describe placing a male and female together in the same cage; 'mating' to mean that copulation has occured as judged either by the arrival of a litter or the finding of a vaginal plug.)

The length of the oestrous cycle was determined by the vaginal-smear technique, details of our accuracy with this technique being given elsewhere (Fowler & Edwards, 1957). Smears from some mice were recorded daily for up to 10 consecutive days. Single smears from other mice were taken as an estimate of the number of mice in oestrus at a particular time. The frequency of oestrus was also measured by pairing females with outbred males of high sexual activity, the females being examined daily for vaginal plugs. This method will be referred to as 'mating performance'.

Ovulation was studied by autopsying mice at approximately 18 hr. after mating. The autopsies were carried out during late afternoon to ensure that ovulation was complete. The recently ovulated eggs were counted, and many of them were examined under the phase-contrast microscope to ensure that fertilization was normal. The number of eggs ovulated was related to the weight of the animal at ovulation.

Other mice that had mated were allowed to carry their foetuses to late stages of gestation or to full term. The proportion of mice with implanted embryos could thus be compared with the proportions which mated and ovulated. Embryonic mortality during pregnancy and parturition was estimated by comparing the mean number of eggs ovulated with mean litter size (living offspring only). Foetal mortality after implantation was measured by counting the numbers of living and resorbing foetuses in mice autopsied during late pregnancy. Foetuses which die between 8 and 11 days' gestation can usually be recognized as a 'mole' during late gestation (Fortuyn, 1919; Huggett & Pritchard, 1944); those which die earlier might leave no trace and thus be excluded from our estimates.

Some females were treated with exogenous hormones. The intraperitoneal injection of pregnant mares' serum (PMS) followed after an interval of 40 hr. by human chorionic gonadotrophin (HCG) induces oestrus and ovulation in adult mice (Fowler & Edwards, 1957). The number of eggs ovulated was found to be largely controlled by the amount of PMS injected. The method was adapted to obtain an estimate of the amount of follicle-stimulating hormone (FSH) secreted by the five lines. It is generally assumed that the number of eggs ovulated during natural oestrus is controlled by the amount of endogenous FSH available. The

mean number of eggs ovulated after natural oestrus was therefore compared with the numbers ovulated after different amounts of PMS, in order to estimate the amount of endogenous FSH.

As will be shown later, many *NS* mice had no implanted embryos after natural mating. Some of these mice were therefore given 2 mg. progesterone in sesame oil daily from day 2 to day 12 inclusive after mating in attempts to maintain their pregnancies (see Smithberg & Runner, 1956, 1957). Other *NS* mice were given PMS and HCG to induce mating and ovulation, and were likewise treated with progesterone.

RESULTS

Fertility in Strain N

The fertility of paired mice

The fertility of pairs of mice of the large, control and small lines, judged by the proportion which produced litters after a period of 2–3 months, is shown in Table 1. Control mice were uniformly fertile, whereas a number of pairs in the large and small lines were sterile.

Table 1. *The incidence of sterility and the time of arrival of first litters in pairs of* NL, NC, NS, CL, *and* CS *mice*

Line	Gens.	No. of pairs	Percentage sterile pairs	Time after pairing when first litters arrived (days)				
				20–21	22–23	24–27	28–35	35
Strain *N*								
Large (*NL*)	37–40	43	18·6	3	9	12	9	2
Control (*NC*)	18–25	47	0	15	21	5	3	3
Small (*NS*)	25–31	67	17·9	0	9	21	15	10
Strain *C*								
Large (*CL*)	16–20	36	5·6	14	13	4	0	3
Small (*CS*)	16–19	25	0	6	10	6	2	1

The interval between pairing the mice and the arrival of the first litter is also shown in Table 1. If the mice mated during the first oestrus period of the female, and assuming that the oestrus cycle was normal, then the expected time of arrival of the first litter would be from 20 to 23 days after pairing. Almost all of the *NC* litters arrived within this period, as compared with approximately one-half of *NL* litters. *NS* litters were even more delayed. Previous work has shown that after eleven generations of selection the delay in arrival of first litters in *NS* mice was already evident, though not so extreme as in the present study, and that no delay existed in line *NL* (Falconer, 1953).

The oestrous cycle

Details of the oestrous cycle during a period of 10 days in large and control mice are given in Table 2. Mice of the large line had a slightly longer cycle than those

Table 2. *The oestrous cycle and mating performance in strain* N *mice*

A. Oestrous cycle judged by vaginal smears

Line	No. of females	No. with cycles	Cycle length (days) 4–5	6–7	8 or more	No. of females recorded once	No. in oestrus
Large (*NL*)	12	10	1	6	3	63	11
Control (*NC*)	6	6	4	1	1	44	10
Small (*NS*)	34*	4	–	–	–	12	0

B. Mating performance

Line	No. of females	Days between pairing and mating 1–2	3–4	5–8	>8	No. not mating
Large (*NL*)	30	9	14	4	1	2
Control (*NC*)	30	11	13	2	3	1
Small (*NS*)	57	3	8	9	11	26

* Smears recorded for 5 consecutive days.

of the controls. Vaginal smears of the small line were discontinued after 5 days: only 4/34 mice had an oestrous smear during this period, the remainder being in dioestrus. Observations on mice recorded for 1 day only, given in the same Table, agree with the above data. The relation between the oestrous cycle of *NS* mice and their age and body weight is shown in Table 3; the presence of a cycle was independent of age or weight.

Table 3. *The oestrous cycle and mating performance of* NS *females in relation to age and body weight*

A. Oestrous cycle

No. of females	Age in days	Weight (g.)	No. of mice with oestrous smear within 5 days
10	42–63	$10{\cdot}9 \pm 0{\cdot}5$	1
9	64–108	$12{\cdot}8 \pm 0{\cdot}4$	1
15	109 and older	$13{\cdot}4 \pm 0{\cdot}5$	2

B. Mating performance

	No. of females	Mean age (days)	Mean weight (g.)
Mated within 16 days of pairing	31	105·2 (Range 58–176)	$13{\cdot}1 \pm 0{\cdot}3$
Failed to mate within 16 days of pairing	26	103·9 (Range 55–163)	$12{\cdot}8 \pm 0{\cdot}4$

The mating performance of females of the three lines, when paired with outbred males for 16 days, is given in Table 2. Both *NL* and *NC* females mated in nearly equal proportions (28/30 and 29/30 respectively) and at approximately the same time. *NL* females had also mated during their first oestrus when paired with outbred males, whereas when paired with *NL* males there was a delay (Fig. 1).

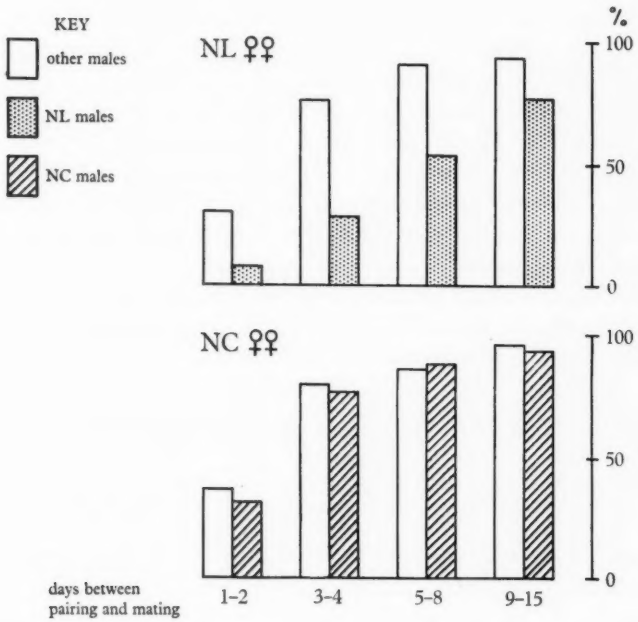

Fig. 1. Accumulative proportion of strain *N* females that had mated to strain *N* or other males at various intervals after being paired. Data on other males taken from Table 2; data on strain *N* males derived from Table 1 (19 days, i.e. the mean gestation period, being subtracted from the interval between pairing and the arrival of the first litters). *NS* males not shown. Many *NS* females failed to produce litters after mating (see text) and the data in Table 1 could therefore not be modified for inclusion.

This difference suggests that the delay noted above (Table 1) was due to *NL* males rather than to the females.

Only slightly more than one-half of the *NS* females mated, many of the matings were delayed, and no relation could be found between mating and age or body weight (Table 3). This evidence confirmed the data from vaginal smears, that selection for small body size had resulted in a lengthened or delayed oestrous cycle.

Male libido

The sterility in some of the *NL* pairs was not due to an abnormal oestrous cycle, nor, as shown below, to foetal mortality during pregnancy. Male sterility was thus suspected as the cause of infertility in this line. To measure the libido of *NL* males,

Table 4. *Mating performance of* NL *males compared with those of another strain when paired with* NL *females*

	No. of pairs	No. mating within 10 days	Females which had not mated exchanged males	No. of pairs	No. mating within 10 days
NL males	14	6	→	2	1
Other males	15	13	←	8	7

one group of *NL* females was paired with *NL* males, and another group with outbred males of another strain. Results are shown in Table 4. *NL* males were obviously less active. When the unmated females were exchanged, most of them mated within 10 days. Furthermore, twelve females from sterile *NL* pairs all mated to outbred males within 5 days of pairing, and all produced litters. Lastly, as shown above, *NL* males were evidently responsible for the delay in the arrival of first litters in *NL* pairs.

NS males from sterile pairs were paired with females of another strain. All of the males sired litters. None of the sterility in the small line during the present investigation was due to male infertility.

Table 5. *Ovulation, mean egg number, and body composition in strain* N *and strain* C *mice*

Strain and line	No. of females		Mean no. of eggs ovulated	Body weight (g.)		Fat in carcass* (g.)
	Mated	Ovulated		Mean	Range	
Strain *N*						
Large (*NL*)	29	29	10·9 ± 0·3	30·1 ± 0·6	24·1 – 36·5	7·2
Control (*NC*)	25	25	7·7 ± 0·3	20·1 ± 0·4	16·2 – 25·0	1·9
Small (*NS*)	32	25	4·5 ± 0·2	13·3 ± 0·3	10·5 – 16·5	1·8
Strain *C*						
Large (*CL*)	22	22	14·8 ± 0·3	35·9 ± 1·9	27·8 – 61·6	3·1
Small (*CS*)	24	24	8·6 ± 0·1	17·6 ± 0·5	14·3 – 22·5	1·2†

	Regression of egg no. on body weight	Ratios of egg no. to body weight	
		Including fat	Excluding fat
Strain *N*			
Large (*NL*)	0·03 ± 0·10	0·36	0·48
Control (*NC*)	0·07 ± 0·15	0·38	0·42
Small (*NS*)	0·08 ± 0·12	0·34	0·39
Strain *C*			
Large (*CL*)	0·01 ± 0·06	0·41	0·45
Small (*CS*)	‡0·49 ± 0·14	0·49	0·52

* Fowler (unpublished)
† Estimates based on males
‡ Significantly different from zero ($P < 0·01$)

Ovulation and fertilization

The proportion of mice that ovulated after mating is shown in Table 5. All of the *NL* and *NC* mice possessed recently ovulated eggs. In contrast, approximately one-quarter of the *NS* mice failed to ovulate. A scatter diagram of the number of eggs ovulated in relation to body weight is shown in Fig. 2. The mean numbers of

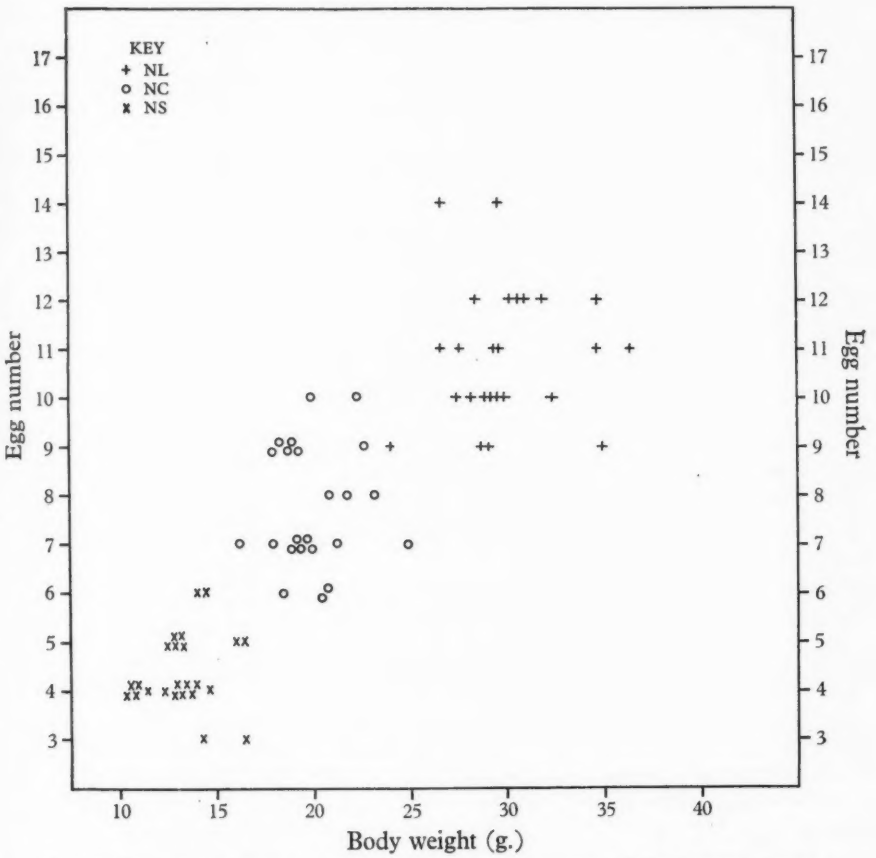

Fig. 2. The relation between the number of eggs recovered after natural mating and body weight in strain *N* females. Each point represents one female.

eggs ovulated, mean body weights, and the regression of egg number on body weight, are given in Table 5. Considerable differences in the numbers of eggs ovulated existed between the three lines, though the egg number per gram of body weight was similar in each line. The number of eggs ovulated was positively correlated with body weight within each line, though the correlations and regressions did not differ significantly from zero. The failure of many *NS* mice to ovulate, and the small number of eggs ovulated, was not due to a deficiency of oocytes, for treat-

Table 6. *Mean numbers of eggs ovulated by strain* N *and strain* C *mice in response to various amounts of PMS and HCG, and estimates of FSH in PMS equivalents*

Strain	Line	No. of mice	Mean no. of eggs ovulated: 6 i.u. PMS 6 i.u. HCG	3 i.u. PMS 3 i.u. HCG	1 i.u. PMS 2 i.u. HCG	½ i.u. PMS 2 i.u. HCG	¼ i.u. PMS 2 i.u. HCG	Mean no. of eggs ovulated during natural oestrus	Regression of egg no. on dose of PMS	Estimates of FSH in PMS equivalents*
N	Large (*NL*)	63	—	20·1 ± 1·6	13·8 ± 1·1	9·4 ± 0·7	—	10·9	3·72	0·6 ± 0·2
	Control (*NC*)	52	—	23·6 ± 3·1	10·7 ± 1·3	9·4 ± 1·3	6·8 ± 1·0	7·7	6·88	0·4 ± 0·1
	Small (*NS*)	61	—	12·4 ± 2·5†	10·2 ± 1·3	10·3 ± 0·9	7·0 ± 1·0	4·5	—‡	⩽ 1/4
C	Large (*CL*)	49	32·3 ± 3·5	21·3 ± 1·8	13·6 ± 1·3	12·6 ± 0·8	—	14·8	3·63	1·2 ± 0·3
	Small (*CS*)	24	—	21·9 ± 4·7	8·8 ± 1·1	8·3 ± 1·6	—	8·6	5·81	0·8 ± 0·2

* See text for description.

† All treated mice ovulated except in this group where 6/20 failed to ovulate.

‡ Regression not calculated because of evidence of suppression of ovulation after 3 and 1 i.u. PMS. Moreover, considerable extrapolation would be required to reach the number of eggs ovulated during natural oestrus.

ment of these mice with gonadotrophins (see below) induced the ovulation of large numbers of eggs. In each of these lines, more than 95% of the eggs recovered after natural mating were fertilized.

The injection of PMS and HCG was used to estimate the amount of FSH secreted by mice of the three lines. Amounts of PMS between $\frac{1}{4}$ and 6 i.u. were injected, and the mean numbers of eggs ovulated at each dose level are given in Table 6. The number of eggs ovulated at each dose level was independent of body weight, both within and between lines, and of the stage of the oestrous cycle of the mice when PMS was injected. The relationship between egg number and dose of PMS was approximately linear in all lines except *NS*, where there was evidence of excess of PMS in the injections (see below). The regression of the numbers of eggs ovulated on dose of PMS was therefore calculated. The amount of PMS equivalent to the mean number of eggs ovulated during natural oestrus could then be derived from the regression equation. These equivalents are given in Table 6.

Small mice evidently received too much PMS when 1 or 3 i.u. were injected, for some failed to ovulate and others ovulated very few eggs. Excess gonadotrophin is known to cause premature luteinization of follicles, thereby suppressing ovulation (Noble, Rowlands, Warwick & Williams, 1939).

Maternal factors and implantation of the embryos

Control mice evidently had little difficulty in maintaining their pregnancies to and beyond implantation (Table 1). Similarly, large mice also maintained their pregnancies with little difficulty, for 28/30 females with vaginal plugs gave birth to litters.

In the small line, only 14 out of 34 females autopsied eight or more days after mating possessed implanted embryos. This degree of infertility could not be explained by failure to ovulate, for less than one-quarter of the mice failed to ovulate after natural mating (Table 5). Six non-pregnant mice autopsied 8 days after mating had no active corpora lutea. Seventeen mice of the small line were therefore given 2·0 mg. progesterone daily from day 2 until day 12 after mating. At autopsy on day 12, the proportion of mice with implanted embryos was considerably increased (Table 7B). The mean number of implanted embryos was not significantly higher.

A further thirty-nine mice of the small line, including eighteen which had failed to mate during 16 days of pairing with outbred males, were given 1 i.u. PMS and 2 i.u. HCG to induce mating and ovulation. Thirty-two of them mated, and these were divided into two groups: one received progesterone, the other received no further treatment. Progesterone treatment increased the proportion of mice with implanted embryos (Table 7C), no significant difference being found between the mean numbers of embryos implanted in the two groups. We have no evidence of the effect of progesterone on pregnancy in *NL* and *NC* mice induced to mate by exogenous gonadotrophins, but other data (Fowler & Edwards, 1960) have shown that fertile mice show no increase in fertility when given progesterone. The data from these *NS* mice were therefore considered to support the observations on mice

Table 7. *The proportion of* NS *mice with implanted embryos, and the mean number of embryos implanted*

A. Mice autopsied at various stages of pregnancy after mating during natural oestrus

	Time when autopsied (days gestation)			
	8–10	11–14	15–18	Total
Number of mice	11	13	10	34
Number with implanted embryos	5	6	3	14
Percentage with implanted embryos	45·5	46·2	30·0	41·2
Number resorbing all embryos	0	1	0	1

B. Comparison between untreated mice and those given progesterone after natural mating

	No. of mice	No. with implanted embryos	% with implanted embryos	Mean no. of implanted embryos
Natural mating, no further treatment	34	14	41·2	4·3 ± 0·3
Natural mating, 2 mg. progesterone from day 2 to day 12	17	14	82·4	4·9 ± 0·3

C. Comparison between gonadotrophin-treated mice given no further treatment and those given progesterone

	No. of mice	No. with implanted embryos	% with implanted embryos	Mean no. of implanted embryos
Gonadotrophins and no further treatment	17	6	35·3	9·2 ± 1·8
Gonadotrophins, 2 mg. progesterone from day 2 to day 12	15	13	86·7	7·2 ± 1·1

that mated during natural oestrus, i.e. that some mice of the small line fail to secrete adequate amounts of progesterone after mating.

Foetal mortality and mean litter size

Usually only a small proportion of foetuses die after implantation, although all foetuses are resorbed in a few mice. In control and large lines, there was no evidence of the resorption of all foetuses, for almost all mice carried their embryos to full term. In line *NS*, mice were autopsied at various intervals during pregnancy, and the proportion with implanted embryos after 15 days was lower than those autopsied earlier (Table 7A). This difference was probably due to sampling, however, for foetuses which die after implantation can usually be detected in the uterus until parturition. Nevertheless, one of the nine pregnant mice autopsied between 11 and 14 days' gestation was resorbing all of its foetuses.

The proportion of foetuses dying in pregnancies maintained to full term was estimated from the difference between the mean number of eggs ovulated and mean litter size (Table 8). Mortality was highest in the large line, and lowest in the small line. The proportion of foetuses which died after implantation was found

Table 8. *Estimates of embryonic and foetal mortality in the selected lines*

Strain and line	Mean no. of eggs ovulated	Mean litter size	Total mortality (%)	Foetuses dying after implantation*	
				Numbers	Percentage
Strain *N*					
Large (*NL*)	10·9	7·9 ± 0·5	27·5	15/116	12·9
Control (*NC*)	7·7	6·7 ± 0·4	13·0	—	—
Small (*NL*)	4·5	4·0 ± 0·2	11·1	3†/27	11·1
Strain *C*					
Large (*CL*)	14·8	9·2 ± 0·6	37·8	—	—
Small (*CS*)	8·6	6·2 ± 0·3	27·9	—	—

* Dashes indicate no observations
† Excluding one female resorbing all foetuses

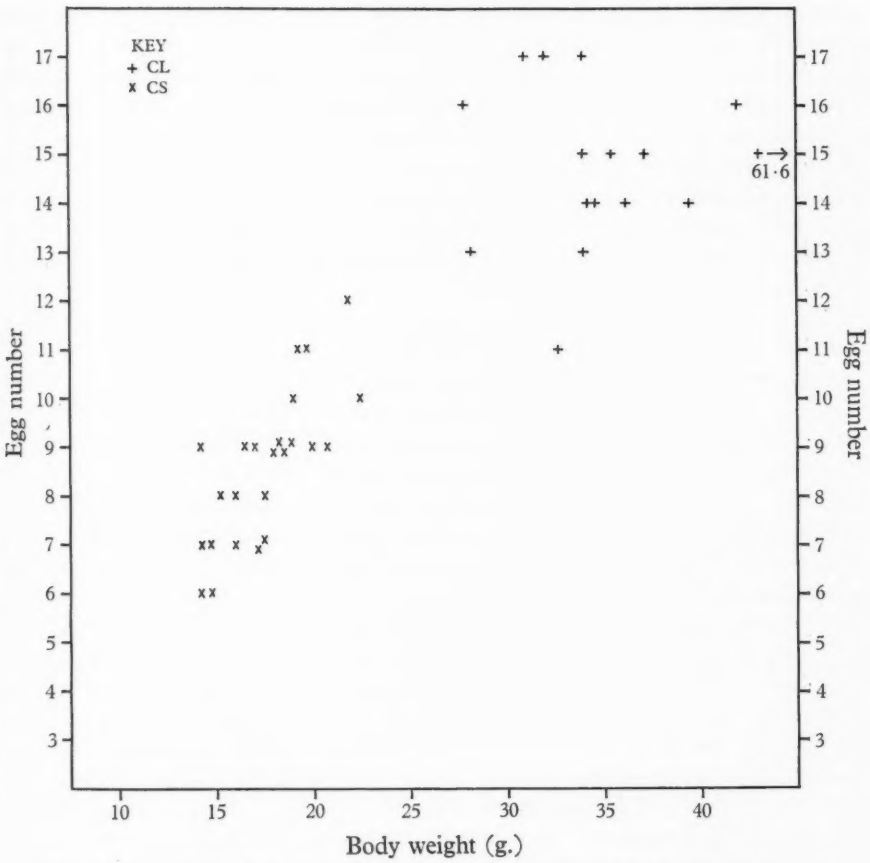

Fig. 3. The relation between the number of eggs recovered after natural mating and body weight in strain *C* females. Each point represents one female.

by autopsying *NL* and *NS* mice during late gestation. Results are given in Table 8, the incidence of resorbing foetuses being similar in the two lines.

Fertility in Strain C

The oestrous cycle of *CL* and *CS* females was normal as judged by the proportion of females giving birth to litters within 25 days of pairing (Table 1). Ovulation was also normal, all mated mice having recently ovulated eggs in the ampullae of the Fallopian tubes (Fig. 3 and Table 5). In the large line, the number of eggs ovulated was not significantly correlated with body weight. In contrast, the correlation in *CS* mice differed significantly from zero (Table 5).

In relation to body size, more eggs were ovulated by females of strain *C* than by those of strain *N*. Differences in the egg-number/body-weight ratios appeared to be mainly due to differences in the amount of fat in the carcasses of the two strains (Table 5). Estimates of the amount of FSH secreted by *CL* and *CS* mice are given in Table 6; the estimates (1·2 and 0·8 PMS equivalents in *CL* and *CS* respectively) were higher than those for strain *N*.

No sterility was observed in *CS* pairs, and only two out of thirty-six *CL* pairs failed to produce litters. Estimates of the incidence of foetal mortality between ovulation and birth in the two lines are shown in Table 8. More than 35% of *CL* embryos died during pregnancy.

The major differences in fertility between strains *N* and *C* were: the absence of any sterility in the males and females of strain *C*, the positive correlation between egg number and body weight in line *CS*, and the higher incidence of foetal mortality in *CL* mice.

DISCUSSION

Different factors were responsible for the infertility in the large and small selected lines of strain *N*. In the large line, the low libido of certain males led to total sterility, or, if less extreme, to delay in the arrival of first litters. The incidence of embryonic mortality between ovulation and birth was also higher in this line than in control and small lines. The higher rate of embryonic mortality was probably due to the presence of the larger number of embryos at implantation (Bowman & Roberts, 1958) rather than to differences in maternal capabilities or variation in embryonic vitality.

The factors causing sterility in the small line of strain *N* strongly suggest a deficiency in gonadotrophin secretion. Thus, the absence of oestrous cycles and failure to ovulate after mating imply deficiencies in circulating follicle-stimulating and luteinizing hormone respectively. Moreover, these mice evidently secreted considerably less FSH than $\frac{1}{4}$ i.u. PMS equivalent, which was lower than the amount estimated in mice of the other lines. The levels of prolactin in many mice of the small line also appeared to be inadequate to maintain functional corpora lutea, and the absence of luteal secretions, which was undoubtedly the cause of failure at implantation, could be remedied by exogenous progesterone.

Selection for small body size has thus apparently impaired the secretion of

gonadotrophins by the anterior pituitary. The decreased growth-rate of these mice might also be due to hypo-functioning of the anterior pituitary, leading to insufficient secretion of growth hormone. A similar conclusion was drawn by Baird, Nalbandov & Norton (1952), who reported that the pituitaries of pigs selected for low rates of gain contained less growth hormone per unit tissue than those selected for high rates of gain. Preliminary experiments (unpublished) indicate that small mice will respond to exogenous growth hormone if sufficiently high doses are given, high doses probably being required because of the species specificity of growth hormones (e.g. Li & Papkoff, 1956; Li, Papkoff & Jordan, 1959).

If increasing sterility accompanies reduced growth-rate, limits will be imposed on the effectiveness of continued selection. Mice which are sterile or semi-sterile will leave few or no offspring for subsequent generations. Falconer (1955) has shown that selection in *NS* mice was impeded in this way: the smallest of the selected mice left fewer offspring than the less small. Extreme sterility could be overcome by ovary transfer, or by inducing oestrus and ovulation with gonadotrophins and then transferring the eggs into fertile mice. Sterility may also limit the effectiveness of selection for large size, if the low libido of some males was due to considerable fat deposition.

In contrast to strain *N*, infertility in pairs of strain *C* mice was negligible. Strain *C* had not reached such an advanced stage in the response to selection as strain *N*, and may become infertile in later generations. A significant positive correlation between egg number and body weight could only be established for line *CS* out of all five lines. Egg number may be correlated with body protein rather than with total body weight, for *CS* mice contained little fat, and the egg-number/body-weight ratios were similar for both strains when fat was excluded. This effect of fat was especially notable in the two large lines; the largest mice of these lines, i.e. those which probably contained the greatest proportion of fat (Fowler, 1958), ovulated the same number of eggs as mice weighing 10–20 g. less.

Estimates of the amount of FSH secreted by mice of strain *C* were higher than those of strain *N*. The level of FSH secretion might be determined by the size of the pituitary (Ladman & Runner, 1959). Moreover, mice of strain *C* deposit more protein than those of strain *N* (Fowler, 1958), and the amount of protein deposited during growth is probably regulated by the amount of pituitary growth hormone available (e.g. Li & Evans, 1948; Greenbaum & McLean, 1953). Inherited differences in the rate of deposition of protein and in the numbers of eggs ovulated indicate a higher level of pituitary activity in strain *C*.

SUMMARY

The fertility of two unrelated strains of mice (strains *N* and *C*) which had both been selected for large and small body size has been studied.

The fertility of pairs of mice in the large or small lines of strain *C* was unimpaired by selection. In strain *N*, some of the pairs in the large and small lines, but not in the control line, were sterile. Sterility in the large line was due to the low libido of

the males, and not to female infertility. Sterility in the small line was probably due to hypo-functioning of the anterior pituitary of some females: the oestrous cycle was delayed or absent, some mice failed to ovulate after mating, and a high proportion of those mating had no implanted embryos at 12 days' gestation. Oestrus and ovulation could be induced in mice of the small line of strain *N* by exogenous gonadotrophins, and the proportion of mice with implanted embryos was considerably increased by progesterone supplements.

The number of eggs found after natural mating was considerably higher in large mice than in small mice, and was significantly correlated with body weight in the small line of strain *C* only. The egg-number/body-weight ratio was higher in the lines of strain *C* than in those of strain *N*, though the ratios were similar when carcass fat was subtracted from total body weight.

The amount of endogenous follicle-stimulating hormone secreted by the mice of the five lines was estimated by inducing ovulation with various amounts of exogenous gonadotrophins, and comparing the number of eggs found after each dose with the mean number ovulated after natural mating. Estimates of the amount of follicle-stimulating hormone secreted by mice of strain *C* were higher than those for mice of strain *N*.

Differences in the rates of growth and in the numbers of eggs ovulated after natural mating indicate a higher level of pituitary activity in strain *C* than in strain *N*.

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Survey of line crosses in a Brown Leghorn flock

1. EGG PRODUCTION

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As a preliminary to more specific studies of certain aspects of hybrid vigour in the fowl, some indication was needed of the combining abilities of the various isolate strains of the Centre's Brown Leghorn flock. A number of individual crosses had been made earlier, but they were mainly from single-sire matings (e.g. Greenwood & Blyth, 1951; Blyth, 1952*a*), and the effect of interyear variations among them made comparisons unsatisfactory. On the other hand, accommodation was insufficient to allow of testing all eight lines together, so the plan adopted was to set up a series of cross-matings including as many lines as possible, having regard to the minimum number of pullets per cross that could be expected to yield useful information. The resultant test is based on a set of 6×6 strain matings, with an average of just under 18 pullets housed per reciprocal group. The immediate purpose of this initial survey was to determine how far the characteristics of the parental strains were expressed in the crosses, and if any particular cross showed an outstanding degree of heterosis which could be further analysed. Subsequent reports will deal with hatchability, growth and body weight in the same experimental material.

The flock from which the birds derive has been closed since 1931. It was built up in the preceding three years from five widely differing sources (three in England, one in Scotland, and one in Denmark). At various times prior to 1939, seven isolate strains were formed from pullets showing diverse outstanding phenotypes. The remaining stock then became the eighth of the lines, all of which are still extant. The latter, line B, selected for both egg size and numbers, is included in the present survey along with L, large egg, I, intensity of production, and N, reduced pausing between laying cycles (a feature which did not persist). These lines, chosen for productive traits, were in existence by 1935. Of the others used, R, increased red pigmentation in the plumage, was formed in 1939. Line W, 'white', was established in the same year from two N hens with white speckled plumage (a simple recessive mutant), but had a bird from line B introduced in 1944. The lines have been subject to mild selection in the direction of their basal traits but, apart from the initial matings, the various degrees of inbreeding which they have undergone are largely incidental to the space allotted to them. The largest line, B, has had annual replacement of about eighty pullets (three to five sires), while I has been maintained at not more than half that number (two to three sires). In the remaining

lines only one to two sires have been used with an annual quota of twenty pullets or less. In the latter groups some mitigation of the consanguinity consequent on such small numbers has occurred through the use of hens of several generations to supply each season's stock. Estimates of the inbreeding coefficients of the lines and crosses are given in Table 1.

Table 1. *Coefficients of inbreeding for lines and crosses* (%)*

Line	L	B	N	W	I	R
L	*64*	14	6	9	8	5
B		*31*	5	11	6	5
N			*57*	21	4	7
W				*62*	4	5
I					*46*	3
R						*74*

* The line coefficients were estimated directly by Wright's method. We are indebted to Mr A. G. Cock for the figures for the crosses; they were obtained by calculating coefficients of inbreeding for hypothetical matings of sets of early line ancestors, and weighting them according to the product of the proportions in which each pair of ancestors now contribute to their respective lines.

Breeding was carried out by artificial insemination. Ideally each sire would have been mated to representatives of all six strains but, with low semen production in some, this was not always possible. The plan was operated as far as practicable and only two sires were used with single females. The total number of parents included in the survey were:

Lines	L	B	N	W	I	R
Sires	5	3	9	6	4	7
Dams	26	24	27	28	23	23

Chicks were hatched at 5-weekly intervals in February and March 1958. This was about a month earlier than usual, but designedly so, to give the pullets an opportunity of entering production before the season of rapidly decreasing day-length which introduces marked genotype-environment interactions into maturity variations. Up to 6 weeks of age they were reared in battery brooders in mixed batches but separated into three rough-size groups. Later the females were transferred to floor pens in an intensive house where they remained until the fastest-developing individuals were close to maturity. Half the population was then moved to individual cages in a battery house, and the remainder rearranged in twenty-bird pens. All the mating groups were divided as evenly as possible, full-sibs being allocated to each location, and pullets of each group distributed randomly over pen and cage positions.

The pens were well lit by continuous roof lighting, but the battery house was considerably darker, daylight entering through small recessed windows in a flat roof. Extra winter lighting was supplied in both houses from October to March, the day being increased from 11½ to 12 hours over the last three months of the year. Mash was fed *ad lib.* in the pens with a scratch feed of mixed grain in the

evening. The cage birds had the same total food formula as an all mash diet till the beginning of May when alterations in the Centre's feeding plans necessitated a change to pellet feeding.

Since the lines derive from the same foundation stock, they represent segregate samples of the same gene pool and, along with the crosses, they can be expected to give some idea of the level and variety of performance that can be obtained by reassortment of genotypes within a particular population of fowls. With all the line and F_1 samples treated alike, it should be possible to regard the group mean differences as mainly attributable to the line genotypes they contain. Thus, comparison of production traits in the contemporary samples of lines and crosses made it possible to examine the position with regard to heterosis in the stock, to see if it occurred in all strain crosses, or was mainly restricted to particular lines or specific matings.

In the descriptions and analyses which follow, the term cross refers to the combined samples from the reciprocal matings of two lines. Where individual reciprocals are mentioned the convention has been adhered to of putting the female line first, e.g. BN refers to the mating B ♀ × N ♂. In the tables giving mean performances of the progeny groups, the matings have been ranked in order of the egg size normally characterizing the lines; for two, N and W, which are very similar for the character, the line weights have changed ranking in the survey samples.

RESULTS

Sexual maturity

Despite the intention of bringing the pullets into lay before the season when marked interaction with environment was likely, the mean maturity ages for the separate progenies showed a wide dispersion, with a range of the order of 10 weeks for both lines and crosses. In pens, the line means run from 174 to 249 days, and those of the cross groups from 150 to 235 days (Table 2). The cage samples are only slightly less variable, so that the general hybrid advantage of 18 days earlier in pens and 13 in cages means little without some knowledge of its content.

Ages at sexual maturity are not very different in the two locations; means of all

Table 2. *Mean age at sexual maturity in days*

	Pens							Cages						
Dam / Sire	L	B	N	W	I	R	Mean*	L	B	N	W	I	R	Mean*
L	*178*	166	174	175	159	167	168	*188*	160	189	186	179	157	174
B	167	*174*	198	191	164	171	178	162	*173*	215	190	181	167	183
N	173	176	*208*	206	169	178	180	172	183	*193*	223	166	180	185
W	180	235	199	*249*	186	178	196	174	185	192	*243*	194	175	184
I	156	165	189	207	*183*	178	179	152	165	194	196	*166*	173	176
R	150	170	167	199	162	*184*	170	153	160	160	189	155	*181*	163
Mean*	165	182	185	196	168	174	178	163	171	190	187	175	170	178

* Crosses only.

cross groups show caged birds to be less than 1 day earlier. This is perhaps not surprising since they were not separated until the most advanced individuals were at point of lay. However, the hybrid progenies of B and R dams laid slightly earlier in cages, as did those of W, I and R sires. The B-dam and W-sire differences of 11 days are entirely due to one extreme cross in pens (BW) which matured 66 days later than the other offspring of B dams there, and 50 days later than its cage equivalent; other differences for sire and dam means did not exceed 1 week. Of the pure lines, L was 10 days later in cages while N and I were 15 and 17 days earlier respectively: even the largest of these three differences is below the 5% level of significance, so it is unnecessary to look beyond sampling error for their source; the last two lines are of small body size, and peck order in the pens may have contributed to the location difference.

An analysis of variance of maturity ages of hybrids confirms that there are marked differences among them (Table 3). Interactions with location occur in

Table 3. *Sexual maturity: analyses of cross, reciprocal and location differences*

	All data		Excluding B-W groups	
	d.f.	Mean square	d.f.	Mean square
Locations	1	83	1	164
Crosses	14	6753**	13	5765**
Reciprocals	15	1555	14	1426**
Location × Crosses	14	639*	13	225
Location × Reciprocals	15	713*	14	379
Error	398	314	366	286

*$P = < 0{\cdot}05$, **$P = < 0{\cdot}01$ throughout all Tables.

both crosses and reciprocals, and, tested against its discrepance, the variation due to reciprocal differences does not attain significance. Both location interactions depend on the presence of the atypically late BW pen sample, for they disappear when the progenies of the intermatings of lines B and W are excluded from the analysis. This allows the mean square for reciprocals to be compared with that for error; on this basis the F ratio becomes highly significant with, or without, the B-W groups. Apart from the latter, five of the other fourteen crosses appear to contribute individually to the inequality of reciprocal behaviour, but even so, the influence of the line characteristics is demonstrable when mean cross maturity ages are compared with the average of their parental means: for the combined pen and cage data there is a regression of $0{\cdot}718 \pm 0{\cdot}15$ of cross on mid-parent value, and a correlation of 0·793 between them. Although this accounts for some 60% of the cross variance, adjustment for this covariance is not sufficient to reduce the remainder mean square below a significant value when tested against error (Table 4). Exclusion of the B-W progenies does not alter the situation and yields slightly lower figures for b and r.

Table 4. *Cross means. Sexual maturity: adjustment of mean square for covariance with mid-parent estimate. All data* (b = 0·7181 r = 0·7926)

	d.f.	Mean square
Regression	1	1956·8**
Remainder	13	89·1**
Error adjusted for analysis of means	398	10·3

The line mean differences in cross performance have been analysed by the reciprocal sums and differences method of Yates (1947), and the results are summarized in Table 8 along with the corresponding data for egg production. As was to be expected from the correlation with mid-parent values, average line differences in sexual maturity are highly significant. There is no indication that dams and sires of the same line are contributing unequally to the effect; the significant reciprocal differences exposed above fall in the remainder portion of their variance which is significantly greater than that for crosses within lines.

In addition to this divergence between reciprocals, the general hybrid advantage in sexual maturity covers a considerable amount of variation among individual groups. Nevertheless, all but two of the pen samples (NB and BW) showed some heterosis in that they matured earlier than the relevant mid-parent estimate. In cages, four were later than this, and the same number approximated to it.

Egg number

Production has been considered mainly on survivors' records. It is possible, and indeed demonstrable, that particular causes of mortality may affect the hybrids unequally. Location effects, however, have made it necessary to examine the pen and cage data separately and, with the initial small numbers in the samples, any chance incidence of non-specific mortality might materially distort the results. Accordingly all birds dying during the laying cycle, or obviously unhealthy, have been eliminated from the detailed analysis. This left a population of 558, of which 100 were purebreds. Comparable records for them are available up to 500 days of age.

The first pullets commenced to lay in July, and in the next few months the wide differences of maturity ages were strongly mirrored in the production records. At first, it seemed likely that the earliest laying crosses would retain their advantage over the whole cycle, but differences in inherent laying rate (clutch size) began to interact with the maturity effects to change the ranking: in pens, crosses of L and R, which along with those of L and I, and of B and I, had been among the top producers till the end of September, had only average records to 500 days; B and I crosses remained in the lead while the other pair of reciprocals finished intermediate between them.

Cross production to 500 days averages 50 eggs better than the parental lines in pens, but there are wide differences among the F_1 yields, which range from 186 to 274 eggs (Table 5); taken as a whole, the differences in mean performance are not

Table 5. *Egg production to 500 days**

Dam	Pens							Cages						
	L	B	N	W	I	R	Mean†	L	B	N	W	I	R	Mean†
Sire														
L	*128*	228	192	189	230	223	212	*123*	206	182	169	178	185	184
B	193	*214*	214	201	251	250	222	201	*192*	175	198	196	207	195
N	214	224	*165*	200	244	201	217	184	183	*164*	170	207	188	186
W	191	186	195	*122*	208	212	198	168	176	193	*113*	190	188	183
I	233	274	214	208	*202*	223	230	202	219	182	189	*209*	215	201
R	215	227	220	206	243	*168*	222	213	214	208	186	220	*153*	208
Mean†	209	228	207	201	235	222	217	194	200	188	182	198	197	193

* Differences of 28 eggs for pens and 26 eggs for cages are significant at $P = 0{\cdot}05$.
† Crosses only.

random but show a noticeable parallelism with mid-parent estimates. In cage samples, which average 24 eggs fewer, the situation is somewhat different: there the variability of means is not so great, and the association with parental ranking less evident. The hybrid advantage is reduced to 34 eggs, and in the individual groups it appears to diminish with increases in mid-parent value. The lines themselves appear less affected by the cage environment and averaged only 8 eggs

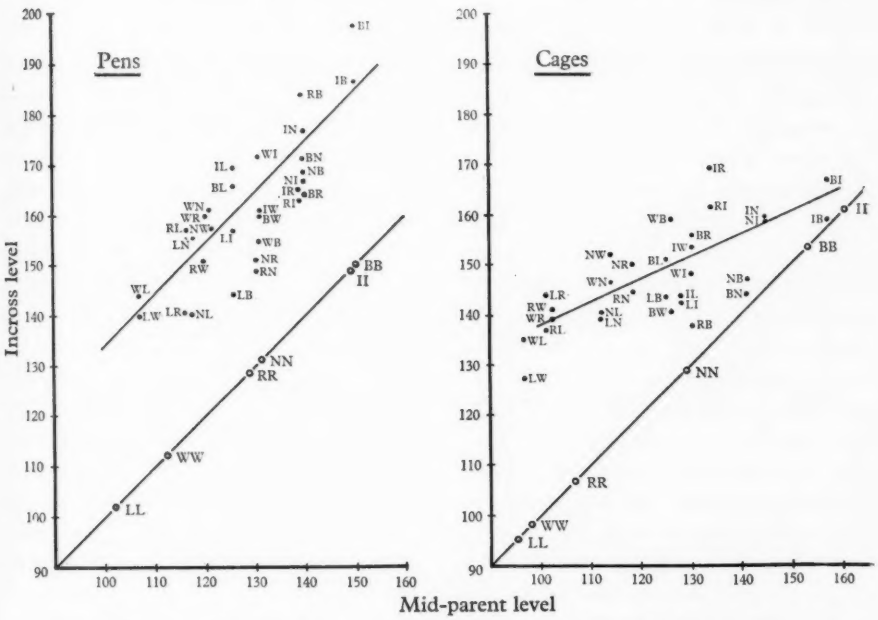

Fig. 1. November through June production of survivors in pens (*left*) and in cages (*right*) plotted against the mid-parent value. The production level of the parent strains themselves are joined by the heavy line and the regressions of cross on mid-parent is shown by the light line.

fewer there. The contrast in behaviour in the two locations is particularly evident when the mean cross records for the months November to June are plotted against the corresponding figures for mid-parents; that is, for that part of the laying cycle from which the sexual maturity effects are excluded (Fig. 1). For the combined reciprocal means (d.f. 13) the regression slopes illustrated are 0·988 for pens, and 0·447 for cages, both significant but also significantly different. For each location removal of this covariance is sufficient to reduce the remainder cross mean square below the 5% level of significance, when tested against that for error (Table 6). For the full 500-day production the association with parental performance, though still marked, does not account for all the variation in cross means.

Table 6. *Egg production cross means: adjustment of mean square for covariance with mid-parent estimate*

	November to June				To 500 days	
	Pens		Cages		Pens	Cages
b:	0·988 ± 0·13		0·447 ± 0·10		0·700 ± 0·14	0·365 ± 0·13
	d.f.	Mean square	d.f.	Mean square	Mean square	Mean square
Regression	1	1816	1	782	3446	936
Remainder	13	32	13	36	139**	113**
Error (adjusted)	199	24	199	21	43	41

Since it seemed to be the variation in sexual maturity that was reducing the relationship of total production and mid-parent level, a general analysis of variance is presented for the November to June period, which excludes it, as well as for the full cycle (Table 7). The cross and location mean squares show variation for both classifications, but the reciprocal differences are only significant in the 500-day one, indicating the effect on the latter of sexual maturity variations. The only significant location interation, that for 500-day crosses, also seems to be a carry-over from the same source: removal of the B-W groups, with the atypically late-maturing BW reciprocal in pens, is sufficient to reduce the F ratio below the 5% level.

Table 7. *Egg production: analyses of cross, reciprocal and location differences*

	d.f.	November–June Mean square	0–500 days age Mean square
Locations	1	18950**	67643**
Crosses	14	3220*	7399**
Reciprocals	15	411	1657**
Location × crosses	14	408	1183*
Location × reciprocals	15	277	402
Error	398	336	636

The influence of age at first laying is also brought out in Table 8, where the line differences for all three criteria are analysed. There the parallel behaviour of the

sexual maturity and 500-day data is in contrast to that of the less variable November to June records. In all three cases there are definite line effects on the hybrids, but none that are consistent for sex of parents. Within lines, however, the reciprocal differences already noted for sexual maturity are also present in the 500-day analysis though their mean square is not significantly greater than that for the cross remainder.

Table 8. *Analyses of line differences in age at sexual maturity, egg production November to June, and to 500 days. Mean squares*

	d.f.	Age at Sexual maturity	Production	
			Nov.–June	500 days
Crosses				
Between lines	5	1174**	559**	1166**
Within lines	9	40	29	58
Reciprocals				
Between lines	5	87	17	37
Within lines	10	133**	29	142**
Error (adjusted)	398	21	22	42

In general, the variations in the full hybrid production to 500 days appear to have three main sources: (1) parental genotype, as evidenced by correlation with mid-parent levels; (2) reciprocal differences originating from variations in sexual maturity, and (3) an additional hybrid gain. With the exception of the aberrant BW pen sample, the behaviour of the crosses in the two locations was reasonably consistent despite the lower level of production in cages.

Egg weight

There is a wide range of egg size in the parent lines, from 46 to 69 g., but the hybrid means remain consistently close to the mid-parent values, averaging less than a gram above the latter (Fig. 2). The largest deviations from mid-parent levels are increases of 3·4 and 3·5 g. in WR and RI crosses respectively. Plotted against egg number, group mean egg weights showed the expected negative trend but there was some irregularity at the lower end of the size scale, and it was noticeable that the groups with the highest yields were near the mean for egg size. In examining the relations between the two characters statistically, the data for crosses were analysed in three ways: (1) as a whole, and then in two subclasses for groups with means, (2) of 55 g. and over, and (3) or less than this. Correlations were computed using March egg weight with October to March production, as well as with estimates of inherent production rate (January to March per cent production with gaps of more than 1 day excluded). Both sets showed the same general picture for all three classifications, although, as was to be expected from previous experience (Blyth, 1952*b*), the significant associations were higher for the rate comparisons: for pens, the total correlation with egg weight for all cross individuals was −0·186 (d.f. 234) for October to March records, but −0·399 (d.f. 228)

for inherent production rate. (The difference in degrees of freedom results from inability to compute rate satisfactorily in a few irregular layers.) For classifications 1 and 2, the cross products for total and group variation were negative in sign; in the third class, they were consistently positive but non-significant. Only in the

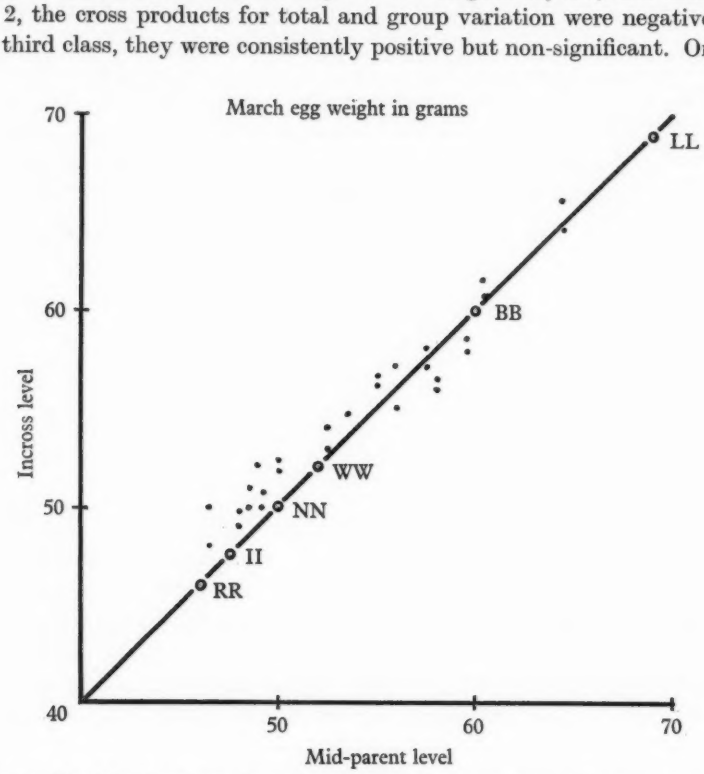

Fig. 2. The March egg weights of birds kept in pens plotted against their mid-parent values.

complete data, and the subclass containing the larger egg-size means, were significant correlations obtained in either pen or cage material. The correlations were slightly higher for the pen birds.

Attempts to split the data in other ways did not suggest any other rational arrangements of relationships. Thus the inference is that there is a limited range of egg size over which weight and numbers are negatively interdependent, and that outside this the association breaks down, or at least alters. The best producers are at the smaller-size end of the covariant range.

Mortality

No reliable picture of pullet mortality was to be expected from this survey: the overall percentages for lines and crosses do not differ greatly, being 13% and 9% respectively for 12 months to the end of June. The figure for lines was almost twice as high in pens (16%) as in cages; the incidence for crosses was the same in

both locations but of different composition: there were more deaths due to fowl paralysis in pens (3% of the cross population there), and cage layer fatigue occurred only in the batteries (3·5%). Most cases of the latter were among progeny of I dams which had 18% deaths in cages but only 8% in pens. N dams, with 25% deaths in both locations, spread very evenly over their cross progenies, had the highest incidence, while only a single pullet died among the offspring of W females.

DISCUSSION

Before discussing the results it may be well to recall the nature of the experimental material used in this survey. The lines are descended from the same foundation stock, and so represent segregate samples of the same gene pool. The numbers in the individual groups are small, particularly where it has been necessary to examine location samples separately, and any deductions relating to specific crosses must remain tentative. Trends over the whole series, however, are likely to be more reliable; they lead to generalizations which may have wider application in similar closed flocks, or even in relation to strains or breed crosses, but authentication of this must await further experiments. Until this is obtained the conclusions reached must be regarded as relevant only to the stock under consideration.

Although the 500-day records of the hybrids tested vary greatly, most are superior to that of the better-parent line in pen samples, and it is striking how far the differences among them reflect parental characteristics rather than an expected diversity of heterotic effects. The relationship is particularly marked when variations deriving from age at sexual maturity are excluded: in the records for November to June, the variation among cross means is completely accounted for by the covariance with mid-parent levels, and the hybrid gain of 34 eggs appears to represent a similar improvement in all crosses. A comparable situation obtains for the less variable cage sample means over the same period, though the hybrid gain is less in the more productive groups.

These two major factors affecting F_1 production rate, parental constitution and hybrid gain, must clearly have been confounded in many previous comparisons. Yet the influence of the former had been suspected as early as 1938 by Knox and Olsen, who believed that better crosses came from better parent stock. Also Harada (1956), working with inbred lines of silkworms, reported regressions of F_1 cocoon weights and filament lengths on mid-parent values very similar to the ones obtained here for pen data. He did not consider the possibility, however, that the association might be complete, but concluded that the hybrid gains decreased proportionately with the increase in parental performance. The 500-day pen records might be interpreted in this way, and, taken alone, the figures for caged groups certainly give grounds for such an inference, for the increases in crosses involving the better parents become insignificant and sometimes negative.

The housing of the birds in two ways has brought in variation in an unexpected form. Lerner (1958) has discussed the general problem of production under different environments; earlier (1955) he expressed the opinion that cross genotypes may

be better 'buffered' than more homozygous ones, and so more resistent to the vagaries of their surroundings. Superficially, the reverse appears to be true here for the lines seem to have withstood the cage conditions somewhat better than the crosses; this applies even when mortality is taken into consideration. In pens, however, even the best of the lines did not reach the mean level of the cross groups, and it may be that they do not actually differ from the latter in their response to the cage environment; they may only appear less affected because they are low in the production series. This makes the virtual absence of location-cross interaction more comprehensible, for the picture for cages of a loss in production that falls most heavily on the higher producers, is partly conditioned by the lesser reduction in line performance. Since the obvious difference between locations relates to the impaired lighting in the battery house, it may be that some relatively simple restriction is then operating, such as insufficient feeding time during the shorter days to allow of a food intake adequate for higher production potentialities to be fully expressed. The change from mash to pellet feeding in May also caused a temporary slackening in production.

Although differences in hybrid performance, once the birds were actually in lay, seemed a matter of 'general combining ability' and additive dependance on parental characteristics, the behaviour of sexual maturity brings in complications which prevent accurate prediction of their relative capabilities. The effect of the single late-maturing BW sample in pens was particularly marked, but its caged homologue fitted quite well into the general patterns of behaviour. Further, the full-sib families in one were equally represented in the other, so there seems little doubt that it is a chance effect. Other reciprocal differences may be of more consequence, but the smallness of the groups makes them particularly subject to sampling errors, and repetitions of the relevant line matings are necessary to test the validity of the differences. Again, in this latitude at least, sexual maturity is considerably affected by interactions between genotype and date of hatch (Osborne, 1952). In the present study the pullets were hatched early, and most of the groups were well in production by the time the disadvantageous conditions of autumn became operative. The late-maturing ones, especially line W and the BW pen sample, however, may have been still further retarded by the rapidly shortening day-length as they approached maturity. This should have brought an error into the mid-parent values containing a W parent, yet the significance of the correlation between cross and parental maturity ages depended largely on these groups. Again, the autumnal environmental effect may have been reflected in pausing among the earlier laying groups, but if so, it did not greatly disturb the general trends brought out in annual production.

While such semi-fortuitous factors could have been responsible for the significant differences between reciprocals, both sex-linkage and maternal effects have been considered with regard to this kind of variation in earlier investigations (Hazel & Lamoreux, 1947). In the present case it is clear from Table 8 that they are not line attributes, and occur only in specific combinations. Their most likely congenital source appeared to be the great differences in line egg size (46 to 69 g.) which might

be producing maternal effects giving rise to interaction with the embryo genotype. No statistical evidence of such intervention could be obtained. Body weight, too, in which parental variations range from 1200 to over 2000 g., but do not entirely parallel those of egg weight, appeared to have no clear connexion with reciprocal behaviour until the set containing the atypical BW group was excluded. Then the differences in mean maturity ages between reciprocals for pen, and pooled location means (but not for cage figures alone), yielded significant correlations (0·62 and 0·52; d.f. 12) with the difference in adult body weights of the parent lines. The evidence is slight; it may be fortuitous, or relate only to variations within a particular gene pool. Nevertheless, the possibility that the way in which two strains of differing size are mated may affect the production of their progeny is a point worth further investigation.

Although the irregularities of variation in sexual maturity detract from the general parent-progeny association for the full records, two of the lines used were very late in maturing, and strains as retarded as line W are unlikely to be retained in commercial flocks. Further, the practice of controlling the time at which pullets enter production, by artificial means, would also tend to reduce these initial differences, and so make the parental production rate a better guide to cross behaviour. It should be emphasized, however, that the results of the present study depend on a comparison of cross production with the mean performance of the parent lines: comparison with the better parent would not lead to so consistent a picture.

The fact that the hybrid gain in pens appears to be a constant for all groups over the greater part of the laying year, is an unexpected phenomenon. It had been supposed that the lines were suffering from inbreeding degeneration, and that cross improvement would be related to the variable, though not extreme, amounts of inbreeding to which they had been subjected or to the consanguinity of the parents. If the hybrid gain represents restitution of inbred loss, why has it not reflected these differences? Is it that the degree of inbreeding is not different enough to create measureable variation, or that the methods of stock replacement used, involving a relatively slow increase in homozygosity over a long period, have resulted in genotypes which are different but yet subadequate to nearly the same extent? As far as the material will allow, this aspect of hybrid vigour will be taken up in a later report, but it is clear that similar analyses of the whole problem are needed using other stocks. The wide inter-line variation in production phenotypes has been advantageous in exposing the role of general factors participating in cross improvement, but their relative importance in more uniform, and genetically different, commercial stocks remains to be determined. The few experiments reported that are in any way comparable with the present survey do not suggest that our results are atypical. Warren (1942), Knox *et al.* (1949) and King & Bruckner (1952) did not get as much hybrid gain with breed crosses though the last-mentioned authors used larger numbers. Maw (1949), with six inter-matings of seven inbred lines, obtained about the same range of variation of cross increases. In Yao's (1959) report, it is not clear whether the 9 months production figures

given refer to birds actually in lay, but if so they provide an interesting comparison with the post-maturity records described here. Reduced to an 8-month value to bring them in line with the November to June figures quoted above, it appears that the parental inbred lines were more degenerate, and that the best of his F_1 groups laid at approximately the same level as the poorest crosses in the present series. Plotted against their mid-parent values, the scatter of his crosses suggested that the hybrid gain was greater for the better parents. When combined with the November to June data, however, a regression on mid-parent of $0{\cdot}79 \pm 0{\cdot}05$ (d.f. 19) was obtained which is within the limits of error of the value $0{\cdot}99 \pm 0{\cdot}13$, calculated for the latter alone.

The importance of parental performance levels in relation to cross records raises the question of the attributes of a good line in this respect. Further investigation of the components of production may hold some clue. It was noted, for instance, that the negative interdependence of egg number and egg weight only extended over the upper half of the egg-weight range, and that the egg size in the best laying crosses was at the lower end of this covariant series, and so near the flock mean. These crosses were also roughly intermediate in adult body size. Whether this represents an optimum for the flock genotype seems a point worthy of investigation. In pens, IN and IR, with smaller egg size, both laid over 240 eggs in 500 days, but none of the groups with means of over 56 g. exceeded 230 eggs. It may be that, in some stocks, egg-size requirements constitute a limiting factor in the search for better egg production.

While the limitations of the present material preclude any accurate assessment of the part played by mortality in contributing to the variations in hybrid production, the general distribution of deaths among the cross groups suggests that it may be not unimportant. Though its effect may be less serious under practical conditions, the situation with regard to sexual maturity also requires further elucidation. Yet, however much these variables contribute to the level of egg production in crosses, it is clear that one essential requirement for successful hybrids is parent stock which are themselves good layers.

SUMMARY

Six isolate lines of long standing, from the Centre's Brown Leghorn flock, were intermated in all possible directions, providing thirty cross and six pure line samples. Half the birds from each line and reciprocal cross were kept in floor pens and the rest caged in an adjacent battery house.

Sexual maturity, survivors' production to 500 days of age, and November through June egg numbers, all showed two main factors contributing to variations in cross performance: (1) a significant association with parental production levels as measured by mid-parent averages, and (2) an additional hybrid gain. For November to June production in pens, this hybrid gain appeared to approach a constant for the cross groups. Additional sources of variation were present in age at sexual maturity.

The 500-day records for the penned hybrids exceeded the line average by 50

eggs. Caged samples did less well and showed an advantage of only 34 eggs. In this location the hybrid gain for individual groups decreased with increases in mid-parent level.

Egg weight varied negatively with egg numbers only in the upper half of the wide egg-size range. The best layers fell at the lower end of the covariant range for egg size.

The importance of improving closed strains may not have been lessened by the expansion of the hybrid side of the poultry industry, for the pattern of relationships of line and cross production in this flock is consistent enough to justify the inference that the best hybrids come from the best-producing parents.

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Effect of inbreeding on ovulation rate and foetal mortality in mice

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INTRODUCTION

The reduction of the litter size of mice resulting from inbreeding was described in two earlier papers (Roberts, 1960; Bowman & Falconer, 1960). The number of live young born in first litters was taken as a measure of litter size, and this was found to decline in a very regular manner as inbreeding increased: at an inbreeding coefficient of 50% the reduction was about $2\frac{1}{2}$ young per litter. Crosses between lines inbred to 50% showed that the reduction was attributable in part to inbreeding in the litters and in part to inbreeding in the mother bearing the litter. The present paper is concerned with the developmental stage at which the reduction of litter size takes place. The reduction could arise from a reduced ovulation rate, an increased loss of eggs or embryos before implantation, or an increased mortality of embryos at any stage between implantation and birth. Dissections of pregnant females were made in order to identify the stage at which the losses from inbreeding take place. The females were dissected 16 days after insemination, and counts were made of (1) the number of corpora lutea, as a measure of the number of eggs ovulated, (2) the total number of implantation sites, and (3) the number of embryos alive at 16 days of gestation. The difference between (1) and (2) indicated pre-implantation losses and the difference between (2) and (3) indicated post-implantation mortality. Further, a comparison of the number of embryos alive at 16 days in the dissected females with the number of live young born to comparable females, allowed to bring their litters to term, provided a measure of perinatal loss, i.e. losses occurring in the last 2 or 3 days of gestation and between the birth and the recording of the litter. Our interest was primarily in the maternal contribution to the inbreeding depression of litter size, i.e. in the stage at which inbreeding of the mother reduces the size of the litter she bears. For this reason most of the comparisons made were between inbred and non-inbred parents, both with non-inbred embryos.

STOCKS AND TECHNIQUES

Three series of dissections were made, all on females of the same basic stock. The dissections and counts of the first series were made by Roberts, those of the second and third series by Falconer. The details of the three series were as follows:

Series I. The mice came from the inbred lines described by Roberts (1960).

Twenty-one independently inbred lines were represented among the females dissected. Five groups of females were arranged according to whether the mother, the father, or the embryos were inbred or non-inbred. The inbreeding coefficients of the mice used in the five groups are given with the results in Table 1. The inbreeding coefficients were either 50% or 59%, or zero.

Series II. The mice came from an experiment on selection for sex-ratio (Falconer, 1954). Nine independently inbred lines were represented among the females dissected. The mice for dissection were 50% inbred and they were mated to males similarly inbred but of a different line. The embryos were thus non-inbred. Comparisons were made with a contemporary group of non-inbred females, produced from line-crosses and mated to similar males from a different line-cross. Thus the only difference between the two groups compared in the series-II dissections was in the inbreeding of the parents.

Series III. The inbred mice were derived from six of the independently inbred lines described by Bowman & Falconer (1960). The females were mated to males from a different line, in the same way as in Series II, so that only the parents and not the embryos were inbred. The coefficient of inbreeding of the dissected females was 63%. The non-inbred mice for comparison came from three sources: a line selected for large litters, a line selected for small litters, and a control line maintained without selection. All three lines had been maintained with minimal inbreeding, and were in the fourth generation of the selection experiment.

All the dissections were made 16 days after insemination, indicated by the presence of a vaginal plug. The uteri were opened and the numbers of implantation sites and of live embryos were noted. Few embryos were found that had died at an identifiable stage of development. For this reason the different stages of post-implantation death were not distinguished in the analysis of the results. The numbers of corpora lutea were counted by examination of the ovaries under a low-power binocular microscope. Exact correspondence between the number of corpora lutea counted and the number of eggs shed was not to be expected, because it was difficult, particularly when the corpora lutea were numerous, to distinguish between one large corpus luteum and two adjacent and partially confluent ones. On the whole the number counted is probably an underestimate rather than an overestimate of the ovulation rate. The corpora lutea counted in some mice of the first series of dissections were compared with egg counts made on a comparable group of females by Dr A. W. H. Braden. The mean number of corpora lutea among 38 females was $10{\cdot}6 \pm 0{\cdot}27$ and the mean number of eggs among 32 females was $10{\cdot}1 \pm 0{\cdot}29$. The close correspondence between the two suggests that, in this series at least, the corpora lutea counts gave a good estimate of the ovulation rate. Among the non-inbred mice of series II and III, 9 out of 74 pregnancies (12·2%) showed an excess of implants over corpora lutea (see Table 3). This, however, is an underestimate of the percentage of errors because about 60% of pregnancies showed some loss of eggs before implantation, and an error of counting would only be revealed when there was no loss. As a very rough estimate we may say that probably about 30% of the counts were too low.

Since, however, we are to compare groups of mice counted in the same way, the bias introduced should not seriously affect the conclusions to be drawn.

There were a few mice with corpora lutea but no implantations. These were excluded, on the grounds that a total loss of eggs would be followed by another ovulation before the dissections were made, so the corpora lutea counted would not represent the eggs that had been lost.

RESULTS

The mean numbers of corpora lutea, implants, and live embryos are given in Table 1. Inspection of these figures shows clearly that inbreeding does not reduce the number of corpora lutea but it does reduce the numbers of implants and of live embryos. Let us, however, examine the results more closely. The five groups of females dissected in Series I are shown separately at the top of Table I. None

Table 1. *Mean numbers of corpora lutea, implants, and live embryos at* 16 *days' gestation* (± *standard errors*). *N is the number of females dissected*

Group	Inbreeding coefficients (%) ♀♀	♂♂	Embryos	N	Corpora lutea	Implants	Live embryos
Series I							
A	50	50	59	27	10·0± 0·34	8·4± 0·48	7·1± 0·48
B	50	50	0	29	9·9± 0·32	7·6± 0·54	6·7± 0·52
C	59	0	0	30	10·2± 0·51	8·8± 0·50	7·8± 0·53
D	0	59	0	28	9·9± 0·26	8·9± 0·37	8·1± 0·52
E	0	0	0	30	10·3± 0·33	9·1± 0·40	7·8± 0·54
A+B+C	50–59	—	—	86	10·0± 0·23	8·3± 0·29	7·2± 0·30
D+E	0	—	0	58	10·1± 0·21	9·0± 0·27	7·9± 0·37
				t=	*0·22*	*1·79*	*1·57*
				P=	*0·9*	*0·1*	*0·2*
Series II	50	50	0	13	10·9± 0·58	8·5± 0·69	7·3± 0·74
	0	0	0	15	11·7± 0·46	11·3± 0·57	9·8± 0·66
				t=	*1·09*	*3·15*	*2·54*
				P=	*0·3*	*0·01*	*0·05*
Series III	63	63	0	17	12·5± 0·82	7·9± 0·82	6·4± 0·80
(H)*	0	0	0	23	10·0± 0·41	9·4± 0·28	8·2± 0·41
(L)*	0	0	0	18	11·9± 0·80	9·9± 0·71	8·9± 0·69
(C)*	0	0	0	18	9·1± 0·29	8·0± 0·42	7·4± 0·47

* H, L and C are the lines selected for high and low litter size and unselected control, respectively.

of the differences between the groups, considered separately, are clear enough to allow us to draw firm conclusions about the different effects of inbreeding in the mother, the father, or the embryos. In particular, the only comparison that contains information about the effect of inbreeding in the embryos (groups A

and B) shows no reduction of the numbers of implants or embryos. The apparent absence here of any effect of inbreeding in the embryos cannot, however, be given much weight because the numbers are not very large and because an increase of the numbers born from crosses between highly inbred lines has often been observed (see, for example, Eaton, 1953). Our chief interest here is in the effect of inbreeding in the mother of the litter, and for this purpose we may combine the groups according to whether the mother was inbred or non-inbred. This gives the same type of comparison as is made in the dissections of Series II and III. The combined groups are given also in Table 1. The numbers of corpora lutea are now almost identical, showing that the ovulation rate is not affected by inbreeding. The numbers of implants and of live embryos are both lower in the inbred females than in the non-inbred, though neither of these differences reach a fully convincing level of significance.

The results of the Series II dissections are quite clear. There is a small but non-significant difference in corpora lutea. Both the implants and the live embryos are fewer in the inbred than in the non-inbred females, and both differences are significant at the 5% level. In the Series III dissections the inbred females have more corpora lutea, but again fewer implants and live embryos. The three non-inbred groups, however, differ significantly between themselves and therefore cannot be combined. These differences were associated with differences of body weight.

The significance tests of the differences between group-means given in Table 1 and also in Table 2 followed the method given by Snedecor (1956, pp. 97–98) which does not assume equality of variance. In fact, the variances of the inbred and non-inbred groups differed significantly in almost every comparison, the inbreds being the more variable. It is not possible, however, to draw genetical conclusions from this fact because the expected changes of the genetic variance at intermediate levels of inbreeding cannot be predicted unless all the variance is additive (Robertson, 1952). For this reason the variances will not be further discussed.

From the three series of dissections we may conclude at this stage that inbreeding does not reduce the ovulation rate as measured by the number of corpora lutea. The reduction of litter size at birth must therefore arise from losses of eggs or embryos either before or after implantation.

Losses

Consideration of the losses of eggs or embryos makes the picture clearer. The pre- and post-implantation losses in the three series of dissections are given in Table 2. The dissections of Series I are here grouped according to the inbreeding of the female parent. The three groups of non-inbred females in Series III are here combined because the differences between them, though still just significant, are much less. All three series agree in showing a greater pre-implantation loss in inbreds than in non-inbreds, but no difference in the post-implantation losses. The significance of the difference of pre-implantation loss does not quite reach the 5% level in Series I, but in Series II and III it reaches the 1% level. There can be

Table 2. *Mean numbers of pre- and post-implantation losses (± standard errors). The percentage losses show respectively the percentage of corpora lutea not represented by implantation sites and the percentage of implants not represented by live embryos at 16 days*

Series	Group	N	Loss: Pre-implantation		Loss: Post-implantation	
I	A+B+C (♀♀ inbred)	86	1·77 ± 0·245	17·6%	1·07 ± 0·179	12·9%
	D+E (♀♀ crossbred)	58	1·12 ± 0·219	11·1%	1·04 ± 0·159	11·5%
			t = 1·99			
			P = 0·05			
II	Parents inbred	13	2·46 ± 0·765	22·5%	1·15 ± 0·355	13·6%
	Parents outbred	15	0·47 ± 0·336	4·0%	1·47 ± 0·390	13·0%
			t = 2·39			
			P = 0·05			
III	Parents inbred	17	4·65 ± 1·010	37·1%	1·47 ± 0·355	18·7%
	Parents outbred	59	1·24 ± 0·237	12 0%	0·93 ± 0·159	10·2%
			t = 3·29			
			P = 0·01			

little doubt that the difference is real in all three series. The conclusion is, therefore, that inbreeding in the female parent increases the loss of eggs or early embryos between ovulation and implantation, but it does not increase the mortality of embryos after implantation.

If the groups of Series I are arranged according to the inbreeding of the male parent, the pre-implantation losses are a little greater with inbred than with non-inbred fathers (1·70 against 1·28), but the difference is insignificant. The pre-implantation loss from inbred mothers is therefore more probably attributable to the females themselves than to a failure of fertilization caused by inbreeding of the male parent.

The distributions of pre-implantation losses are given in Table 3. The higher mean loss in inbreds is due to a larger number of large losses rather than to a difference in the modal loss: in other words, to a drawing out of the upper tail of

Table 3. *Distributions of pre-implantation losses, i.e. numbers of dissections exhibiting each degree of loss. 'Negative' losses represent deficiencies of corpora lutea due to miscounting*

	Number of eggs lost (= excess of corpora lutea over implants)															
	−2	−1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
Series I																
Inbred ♀♀		1	30	18	14	12	5	0	1	0	1	3	1			
Outbred ♀♀	1	1	24	16	7	4	1	3	0	1						
Series II and III																
Inbred ♀♀			6	7	2	2	3	2	1	1	2	1	1	1	0	1
Outbred ♀♀	1	8	23	23	7	5	4	0	0	2	1					

the distribution. The skewed distributions make the exact level of significance of the *t*-tests a little uncertain, but the reality of the difference between inbred and non-inbred females can hardly be questioned.

Nothing has yet been said about the perinatal losses. Not all the groups of dissected females had suitable females available for comparison of the number of young born alive. Those available for valid comparison are shown in Table 4.

Table 4. *Comparison of live embryos at* 16 *days and live young at birth in litters of comparable females*

		Live embryos		Live births	
Series	Parents	N	Mean ± s.e.	N	Mean ± s.e.
I B	Inbred	29	6·7 ± 0·52	107	6·2 ± 0·067
E	Outbred	30	7·8 ± 0·54	147	8·5 ± 0·034
II	Inbred	13	7·3 ± 0·74	21	7·4 ± 0·70
	Outbred	15	9·8 ± 0·66	27	8·3 ± 0·38

The evidence, as far as it goes, points to the perinatal losses being very small, and substantially the same in inbred as in non-inbred mothers.

Connexion between ovulation rate and body weight

The connexion between ovulation rate and body weight has an important bearing on the interpretation of the fact that ovulation rate did not decline with inbreeding. This will be explained below, in the Discussion, but the data are given here. The influence of body weight on the number of corpora lutea was examined in the dissections of Series I. The females were weighed at 6 weeks of age and were mated soon after, so that the ovulation corresponding to the corpora lutea counts took place between the ages of about 7 and 9 weeks. The regression of corpora lutea on 6-week weight was 0·244 ± 0·063 corpora lutea per gram. (The regressions did not differ significantly between the groups and this is the pooled value.) There is, thus, a positive association between body weight and ovulation rate. Comparisons of ovulation rate between inbred and outbred females should therefore take into account any differences of body weight. The mean weights of the females in the three series of dissections are given in Table 5, together with the mean ages at ovulation and the numbers of corpora lutea, from Table 1, to facilitate comparison. It will be seen that the weights of the inbred and outbred females of series I were very nearly the same, and so were the ovulation rates. The inbred females of Series II were 2 g. lighter than the outbred females at the time of ovulation (the weights were not recorded at 6 weeks) and the ovulation rate was lower. In Series III, as in Series I, the mean 6-week weight of the inbred females was not less than that of the outbred females. The differences of weight between the groups were, however, rather larger than in Series I, and the inbred females were substantially older than the outbred ones at the time of ovulation. The comparisons of ovulation rates in this series are therefore less reliable.

Table 5. *Weights and ages of the females dissected*

Series	Group	Corpora lutea	Weight at 6 weeks (g.)	Weight at insemination (g.)	Age at insemination (days)
I	Inbred ♀♀ (A+B)	10·0	21·7	—	53
	(C)	10·2	21·7	—	59
	Outbred ♀♀ (D+E)	10·1	21·4	—	58
II	Inbred ♀♀	10·9	—	27·1	93
	Outbred ♀♀	11·7	—	29·0	95
III	Inbred ♀♀	12·5	22·9	—	103
	Outbred ♀♀ (H)	10·0	20·6	—	59
	(L)	11·9	23·8	—	69
	(C)	9·1	22·0	—	60

DISCUSSION

The fact that the ovulation rate, measured by the number of corpora lutea, was not affected by inbreeding calls for some comment. It would be reasonable to expect that body weight would decline on inbreeding, and, since ovulation rate is positively correlated with body weight, a reduction of ovulation rate on inbreeding might reasonably be expected. In fact, however, the weights of the mice used in this work, except those of Series II, did not decline on inbreeding for the following reason (see Roberts, 1960). The weights of mice, both at weaning and subsequently, are inversely correlated with the number reared in the litter, so that mice reared in small litters are larger than mice reared in large litters. (See, for example, Falconer, 1955.) No adjustment of the litter size at birth was made during the inbreeding of the mice dissected in Series I and III, the mothers being left to suckle all the mice to which they gave birth. But the numbers born declined as inbreeding proceded, and consequently the weights tended to increase. Presumably there was a contrary tendency for the weights to decrease from reduced pre-weaning nutrition and slower growth. These two opposing tendencies counterbalanced each other and the weights remained substantially constant. By this fortunate coincidence we were able to assess the effects of inbreeding on the ovulation rate without the disturbing influence of associated changes of body weight. The mice dissected in Series II, however, were differently treated. Litters were standardized at birth to four young during the inbreeding, and the compensatory effect of declining litter size was here absent. The inbred females were lighter than the outbred females and their ovulation rate was lower, though neither difference was statistically significant.

Thus the conclusion that inbreeding does not influence ovulation rate requires qualification because it refers only to the rather special circumstances where the effect of inbreeding on growth is not apparent. Under other circumstances, which allow body size to decline with inbreeding, the ovulation rate would almost certainly decline too. The conclusion, translated into genetic terms, is that genes that

influence ovulation rate without affecting body size do not show directional dominance, but genes that influence ovulation rate as a consequence of their effect on body size may show directional dominance.

It is interesting to note that the effect of inbreeding on the ovulation rate of pigs appears to be different. The ovulation rate is reduced by inbreeding (Squiers *et al.*, 1952; King & Young, 1957), and this—rather than pre- or post-implantation loss—is the chief cause of the reduction of litter size. One cannot invoke a reduction of body size as an explanation because the ovulation rate was not found to be significantly correlated with body weight (King & Young, 1957). An explanation of the difference between pigs and mice might perhaps lie in their past histories of selection. The directional dominance exhibited by the genes affecting the ovulation rate of pigs may be the consequence of selective pressure in the past directed toward increased litter size; there has probably been much less selection of this sort in the past history of laboratory mice.

Reverting now to mice: the conclusion reached from the work described here is that the reduction of litter size in inbred mothers is mainly due to an increased loss of eggs or embryos before implantation. This loss could be caused by failure of fertilization or by failure of the fertilized eggs to implant. Since the inbreeding of the male did not influence the pre-implantation loss, any failure of fertilization would have to be attributed to the female. Losses attributable to the female might arise from (i) the production of abnormal eggs, (ii) impaired transport of the sperm to the site of fertilization, or (iii) failure of implantation in consequence of an impairment of endocrine function. The first seems unlikely because Braden (1957) found the incidence of abnormal eggs was no higher in inbred than in non-inbred females. The second is possible because only quite a small number of sperm reach the site of fertilization (Braden, 1958) and it would not be unreasonable to suppose that impaired transport might reduce their number enough to leave some eggs unfertilized. Nevertheless, failure of the fertilized eggs to implant, in consequence of an impaired endocrine function, seems to be the more likely cause of the increased pre-implantation loss in inbred females.

SUMMARY

Dissections were made of 16-day-pregnant female mice with the object of discovering the developmental stage at which litter size is reduced by inbreeding. Counts were made of the numbers of corpora lutea, implantation sites, and live embryos, and comparisons were made between females with inbreeding coefficients of 50–60% and non-inbred females. Except in one group the embryos were all non-inbred, so that the comparisons showed the effect of inbreeding in the mother of the litter. No influence of inbreeding in the male parent was found.

The only difference found between inbred and non-inbred females was in the number of eggs or embryos lost before implantation. The greater pre-implantation loss in inbred females was enough to account for the smaller number of young born alive in their litters.

There was no difference between the inbred and non-inbred females in the ovulation rate, measured by the number of corpora lutea, or in the post-implantation mortality of the embryos.

There was a positive correlation between ovulation rate and weight at 6 weeks. For reasons explained in the Discussion, the inbred females did not differ in weight from the non-inbred females. If, under other conditions, the weight declined on inbreeding, the ovulation rate would be expected to decline also.

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Genetics of plasma transferrins in the mouse

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1. INTRODUCTION

Since the development of starch-gel electrophoresis (Smithies, 1955), genetically determined differences in the electrophoretic mobility of certain plasma proteins have been observed in man, cattle (Smithies & Hickman, 1958), sheep and goats (Ashton, 1958; Ashton & McDougall, 1958). In man, such polymorphism has been found to involve at least two independent systems, namely, the haemoglobin-binding proteins or haptoglobins (Smithies, 1955), and the iron-binding β-globulins or transferrins (Giblett, Hickman & Smithies, 1959). A similar situation occurs in the pig (Cohen, unpublished observations).

None of the species in which these polymorphisms have so far been observed is convenient for experimental breeding, and this prompted a search for similar variations in the mouse. Plasmas from individuals of six inbred strains of mice have so far been examined by starch-gel electrophoresis. Of these, five strains (A, C57BL, JU, KL, RIII) were indistinguishable in plasma protein pattern, whilst the sixth strain (CBA) differed by having a group of β-globulins with a raised electrophoretic mobility. Crosses between CBA and C57BL inbred mice indicated that the difference in β-globulin type between these two strains is under genetic control and is determined by a pair of allelomorphic autosomal genes. An Fe^{59} labelling experiment showed that these β-globulins are transferrins.

The work of Thompson, Foster, Gowen & Tauber (1954) came to my notice after the start of the present investigation. These workers, using moving-boundary electrophoresis, examined the serum proteins of seven inbred strains of mice. They described the presence in one strain (E) of a β_1-globulin not present in the remaining six strains (BALB/Gw, LGW, K, RI, S, Z) and presented evidence that this β_1-globulin could be detected in the F_1 progeny of crosses between E and S strain mice. Because of the differences between moving-boundary and starch-gel electrophoresis, an exact comparison of the results of Thompson *et al.* (1954), and those presented here, is not possible. However, from the results of two-dimensional electrophoresis (Fig. 3) it appears that the transferrins are the major constituents of the β-globulin fraction of mouse plasma. It is, therefore, a reasonable assumption that the β-globulins which Thompson *et al.* (1954) found to show hereditary variation were, in fact, transferrins.

The symbol *Trf* is adopted here for the mouse transferrin locus because *Tf*, as used by Smithies & Hiller (1959) for the human transferrin locus, might be con-

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fused with *tf*, which is used in the mouse for the locus 'tufted'. The transferrin phenotype observed in CBA animals will be referred to as TrfA, whilst that in the other strains will be called TrfB. The phenotype of heterozygous animals, such as those derived from matings between CBA and C57BL animals, will be called TrfAB. The corresponding alleles will be called Trf^A and Trf^B.

Table 1. *The phenotypes and their corresponding genotypes*

Phenotype	Corresponding genotype	Reference
Transferrin A (TrfA)	Trf^A/Trf^A	Present communication
Transferrin AB (TrfAB)	Trf^A/Trf^B	
Transferrin B (TrfB)	Trf^B/Trf^B	
Haemoglobin d (Hbd)	Hb^2/Hb^2	Gluecksohn–Waelsch, cited in Russell & Gerald (1958)
Haemoglobin ds (Hbds)	Hb^2/Hb^1	
Haemoglobin s (Hbs)	Hb^1/Hb^1	
Agouti	A^+/A^+ and A^+/a	Grüneberg (1952)
Non-agouti	a/a	

2. MATERIALS AND METHODS

(*a*) *Mice*

The following five standard strains of inbred mice (Report of International Committee on Standardized Nomenclature for Inbred Strains of Mice, 1952) were examined:

A/Fa	F? +36
CBA/Fa	F? +31
C57BL/Fa	F? +31
RIII/Fa-*ro*	F? +29
JU/Fa	F 25

A sixth strain, known as KL, was made in this laboratory from a four-way cross of the A, CBA, C57BL and RIII strains, and it had been inbred by thirty-six brother-sister matings.

The mice were bled ($\gtrsim$0·6 ml.) from a small incision in the caudal artery. The blood was collected in a capillary tube and emptied into a heparinized polyethylene container. Plasma was removed after centrifugation. All mice were more than 6 weeks old when bled.

(*b*) *Electrophoresis*

(i) Horizontal, one-dimensional starch-gel electrophoresis was performed using, with minor modifications, the method described by Smithies (1955). 'Starch-hydrolysed for gel electrophoresis' prepared by the Connaught Medical Research Laboratories, Toronto, Canada, was employed.

The discontinuous buffer system described by Poulik (1957) was found to be the most useful, since in this system the mouse haptoglobin moves more slowly than any of the transferrins, whilst in the continuous borate buffer system (Smithies, 1955) it overlaps the fastest transferrin components. A piece of Whatman 3-mm. chromatography paper was soaked in each plasma sample and inserted into the gel. Sample-slits were always separated from one another by a short length of unbroken gel, and were placed 5 cm. from the edge of the cathodic wick. A stabilized d.c. potential of 6 V/cm. length of gel was applied for about 6 hours, giving an initial current density of about 5 mA/cm^2 of gel cross-section.

(ii) Two-dimensional filter paper/starch-gel electrophoresis (Poulik & Smithies, 1958) was done on specimens of each phenotype.

The gels were stained for protein with Naphthalene Black 12B. An *o*-dianisidine reagent (Owen, Silberman & Got, 1958) was used to stain free and bound haemoglobin.

3. RESULTS

(*a*) *Electrophoresis*

(*i*) *Plasma proteins*

Plasmas from at least six, and in the cases of CBA and C57BL many more, animals from each of the six inbred strains were examined. In no case was any consistent difference observed between animals of similar age and sex within any one strain. Comparison of plasma samples from animals of different strains showed that the CBA strain differs from the other five, which are all alike.

Two-dimensional electrophoresis showed that the difference affects a group of β-globulins. I am indebted to Dr H. Harris of The London Hospital Medical College for an experiment in which Fe^{59} was added to pooled plasmas of each β-globulin phenotype. Autoradiography subsequent to electrophoresis showed that the Fe^{59} was bound only to the variable β-globulins, thus demonstrating that these are transferrins (cf. Giblett *et al.*, 1959). The results of this, together with the results of one- and two-dimensional electrophoresis of CBA (TrfA), C57BL (TrfB) and CBA $\times$ C57BL F_1 (TrfAB) plasmas, are presented in Figs. 1, 2 and 3. The TrfA electrophoretic pattern comprises three components visible as bands in the stained gels. Of these bands, the fastest-moving is very faint, the second-fastest is fairly strong, and the slowest one is strong. The TrfB electrophoretic pattern also comprises three components, all of which migrate more slowly than their TrfA counterparts. Thus the fastest TrfA band is faster than any of the TrfB bands, whilst the second-fastest TrfA band moves at the same speed as the fastest TrfB band. The electrophoretic pattern of the heterozygote (TrfAB) is consistent with that to be expected from a mixture of equal parts of TrfA and TrfB plasma, i.e. four bands are visible, of which the faster two are faint and the slower two fairly strong, all four corresponding in mobility with bands present in the two homozygotes (Figs. 1 and 3).

The Fe^{59} autoradiographic results, however, give no evidence of iron binding by the fastest (faint) component in any of the three phenotypes. Whether this is

due to the relatively small amount of this protein which is present, or to it being a protein other than transferrin, and therefore unable to bind iron, remains to be determined.

No evidence of haptoglobin differences was found among the mouse strains examined.

(ii) *Haemoglobins*

Gluecksohn-Waelsch, Ranney & Sisken (1957) demonstrated by filter-paper electrophoresis the existence of a pair of alternative phenotypes of mouse haemoglobin. These phenotypes were called 'diffuse' (Hbd) and 'single' (Hbs), and were shown to be determined by allelic genes (see Table 1 for nomenclature). Russell & Gerald (1958) have shown that, phenotypically, CBA mice are Hbd, and C57BL mice, Hbs. In the present work, using starch-gel electrophoresis, these findings have been confirmed and extended. The diffuse phenotype has been resolved into three haemoglobin components, and the single phenotype into two. It has proved possible to distinguish the heterozygote (Hbds), the electrophoretic pattern of which is similar to that given by a mixture of equal parts of Hbd and Hbs haemoglobin preparations.

(*b*) *Segregation data*

The CBA and C57BL strains differ in the following relevant ways:

CBA	C57BL
agouti	non-agouti
Hbd	Hbs
TrfA	TrfB

By crossing CBA males with C57BL females, an F_1 generation of twenty-nine animals was reared. These were all phenotypically agouti, Hbds, TrfAB. Thus, since all the F_1 males were heterozygous, sex-linkage of the transferrin locus is excluded. Using the maximum likelihood estimation procedure described by

Table 2. *Classification of 128 F_2 mice for sex, coat colour, haemoglobin and transferrin phenotypes*

Transferrin phenotypes and sex		Haemoglobin and coat-colour phenotypes: Hbd Agouti	Hbd Non-agouti	Hbds Agouti	Hbds Non-agouti	Hbs Agouti	Hbs Non-agouti	Totals	
TrfA	♂	4	2	6	3	0	1	16	40
	♀	5	3	8	3	5	0	24	
TrfAB	♂	5	0	14	6	8	2	35	62
	♀	2	2	9	3	6	5	27	
TrfB	♂	3	2	7	0	1	1	14	26
	♀	2	0	7	1	2	0	12	
Totals		21	9	51	16	22	9		
		30		67		31			128

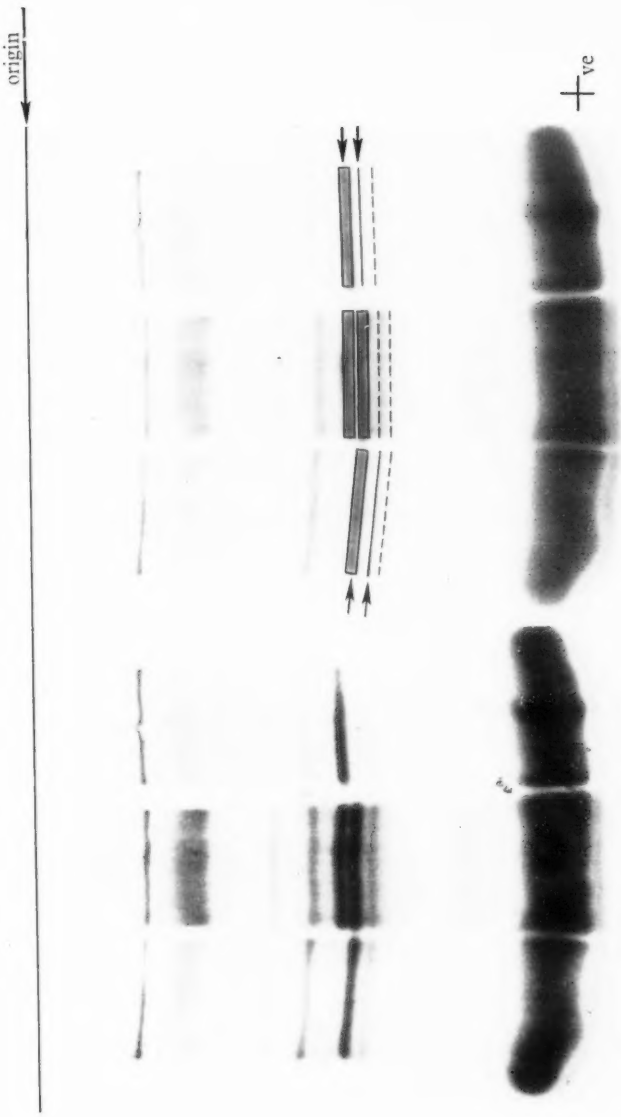

Fig. 1. *Left:* one-dimensional starch-gel electrophoresis of mouse plasma. *Right:* the same, with the β-globulin bands inked in. The arrows indicate those bands for which clear evidence of Fe^{59} uptake has been obtained (see text). (*a*) CBA (TrfA); (*b*) CBA×C57BL F_1 (TrfAB); (*c*) C57BL (TrfB). × 1.

(*Facing p. 434*)

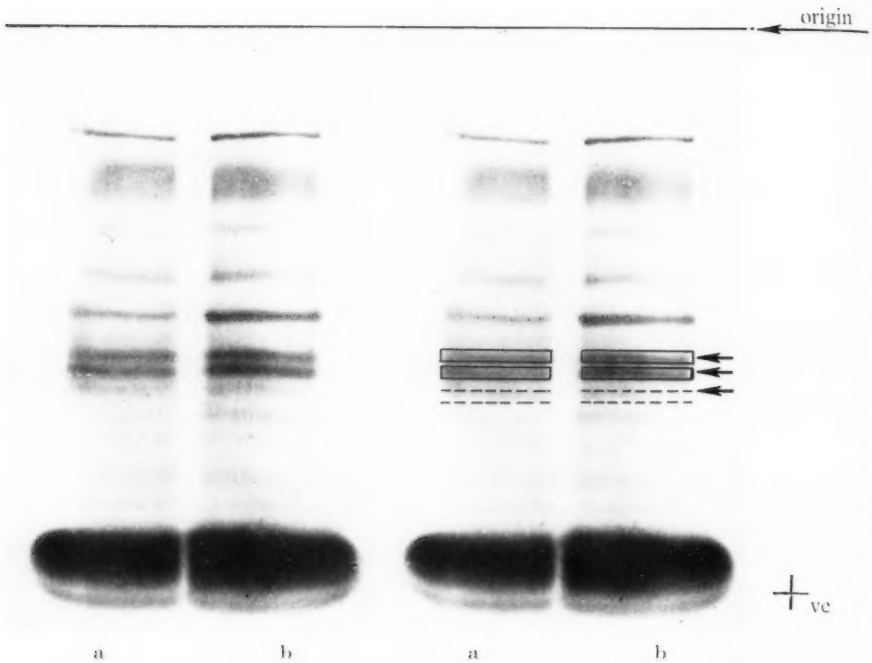

Fig. 2. *Left:* one-dimensional starch-gel electrophoresis of mouse plasma. *Right:* the same, with the β-globulin bands inked in. The arrows indicate those bands for which clear evidence of Fe^{59} uptake has been obtained (see text). (*a*) Artificial mixture of equal parts of CBA and C57BL plasma; (*b*) CBA × C57BL F_1 (TrfAB) plasma. × 1.

Fig. 3. *Left:* two-dimensional filter-paper/starch-gel electrophoresis of mouse plasma. Regions of the filter-paper strips, 3cm. long, and containing the β-globulin zone, were cut out and inserted side by side into the gel. *Right:* the same, with the β-globulin bands inked in. (*a*) CBA (TrfA); (*b*) CBA × C57BL F_1 (TrfAB); (*c*) C57BL (TrfB). × 1.

Carter & Falconer (1951), no evidence for partial sex-linkage was obtained. A total of 128 F_2 and 90 backcross animals were reared and classified. Of the backcross animals, 66 were from $F_1 \times$ CBA, and 24 from $F_1 \times$ C57BL matings. The numbers of animals observed in each phenotypic class are presented in Tables 2 and 3.

Table 3. *Classification of backcross mice for sex, coat colour, haemoglobin and transferrin phenotypes*

Transferrin phenotypes and sex		$F_1 \times$ CBA			$F_1 \times$ C57BL				
					Hbds		Hbs		
		Hbd Agouti	Hbds Agouti	Totals	Agouti	Non-agouti	Agouti	Non-agouti	Totals
TrfA	♂	5	12	17	0	0	0	0	0
	♀	14	6	20	0	0	0	0	0
TrfAB	♂	7	10	17	2	1	2	2	7
	♀	7	5	12	2	0	2	2	6
TrfB	♂	0	0	0	2	0	1	2	5
	♀	0	0	0	1	1	2	2	6
Totals		33	33	66	7	2	7	8	24

From these data χ^2 values were obtained to test the significance of the differences between observed and expected numbers in the segregation of the transferrin phenotypes on the hypothesis that they are determined by allelic genes. Similar calculations were made with respect to the haemoglobin and coat-colour segregations. None of these χ^2 values is significant at the 5% level of probability (Table 4).

Table 4. χ^2 *tests of the segregation ratios*

	Transferrin phenotypes				Haemoglobin phenotypes				Coat-colour phenotypes		
	TrfA	TrfAB	TrfB	Total	Hbd	Hbds	Hbs	Total	Agouti	Non-agouti	Total
F_2 generation											
Obs.	40	62	26	128	30	67	31	128	94	24	128
Exp.	32	64	32	128	32	64	32	128	96	32	128
	$\chi^2 = 3{\cdot}2$, $P > 0{\cdot}2$				$\chi^2 = 0{\cdot}3$, $P > 0{\cdot}8$				$\chi^2 = 0{\cdot}2$, $P > 0{\cdot}5$		
Backcross $F_1 \times$ CBA											
Obs.	37	29	0	66	33	33	0	66	66	0	66
Exp.	33	33	0	66	33	33	0	66	66	0	66
	$\chi^2 = 1{\cdot}0$, $P > 0{\cdot}3$										
Backcross $F_1 \times$ C57BL											
Obs.	0	13	11	24	0	9	15	24	14	10	24
Exp.	0	12	12	24	0	12	12	24	12	12	24
	$\chi^2 = 0{\cdot}2$, $P > 0{\cdot}5$				$\chi^2 = 1{\cdot}5$, $P > 0{\cdot}2$				$\chi^2 = 0{\cdot}7$, $P > 0{\cdot}7$		

The scoring method of Finney (1949), with trial values of 0·5 for p in each case, gives maximum likelihood estimates of the recombination fractions, based on the combined F_2 and backcross data as:

	$p \pm$ S.E.$_p$
Trf : *Hb*	0·47 ± 0·034
Trf : A^+	0·55 ± 0·048

In each case the estimate of p differs from the null value of 0·5 by rather less than its standard error. Thus there is no indication of linkage.

The results of statistical tests of the segregation data are therefore in agreement with the hypotheses that the transferrin phenotypes TrfA and TrfB are determined by a pair of allelic genes that segregate independently of both the haemoglobin locus in linkage group I (Popp & St. Amand, 1959) and the agouti locus in linkage group V (Grüneberg, 1952).

4. DISCUSSION

The genetical results presented here are consistent with the conclusion that the difference between the TrfA and TrfB phenotypes is determined by a single pair of allelic genes. The electrophoretic results, and the findings of Thompson *et al.* (1954) (if applicable), are consistent with the conclusion that the transferrins of Trf^A/Trf^A and Trf^B/Trf^B mice differ in charge, rather than in size. The electrophoretic results also demonstrate that each transferrin allele determines the electrophoretic behaviour of three (or possibly only two) β-globulins with iron-binding activity, and that, in the heterozygote, four (or possibly only three) components with the same activity can be resolved. However, because certain of the TrfA and TrfB components migrate in starch-gel with identical mobilities, the phenotypic pattern observed in the heterozygote cannot be distinguished from that given by a mixture of equal parts of plasma from the two homozygotes. There is, therefore, no compelling reason to postulate the existence in the Trf^A/Trf^B heterozygote of any 'hybrid' transferrin substances. Whether such hybrid substances do, indeed, exist might be determined by more refined analysis of the purified proteins, e.g. by the method of Ingram (1956). Similarly, further analysis at the molecular level might furnish an explanation for the mechanism whereby each transferrin allele appears to affect the electrophoretic mobility of three (or possibly only two) iron-binding proteins, and might help to explain the molecular interrelationships of these proteins. One hypothesis which may be proposed, and for which the difference between the human haemoglobin variants HbA and HbS may serve as a model (Ingram, 1956), is as follows: that each transferrin allele controls an amino-acid substitution which affects the charge, and that the same substitution occurs in each of the iron-binding proteins determined by that gene. In this case, on refined chemical analysis, one would expect to find some sort of homology between Trf^A and Trf^B component products of similar relative position, e.g. between the slowest TrfA and TrfB bands, rather than between bands of identical electrophoretic mobilities, e.g. the slowest TrfA and the second-fastest TrfB band.

Genetically determined variations in the plasma β-globulins have now been reported in man, cattle, sheep, goats, horses, pigs and mice. At least in the cases of man, cattle, sheep, pigs and mice, these β-globulins have been found to be transferrins (Giblett *et al.*, 1959; Harris, personal communication). In man and cattle, sufficient data have now accumulated to show that the frequencies of the various transferrin alleles differ in different populations (Smithies, 1957; Harris, Robson & Siniscalco, 1958; Smithies & Hickman, 1958; Ashton, 1959*a*), and these findings raise the possibility that the various alleles differ in adaptive value. Indeed, evidence has already been presented (Ashton, 1959*b*) showing some effect of transferrin genotype on the fitness of cattle.

It would be of great interest to know what physiological and genetic factors underlie the present polymorphism of so many mammalian species with regard to such characters as transferrin type. Inquiry into the nature of the physiological factors involved could probably be pursued conveniently using cattle as experimental animals. However, research into the nature of the genetic factors would be hampered in man and cattle by their long generation-time and by the impossibility or difficulty of carrying out experimental breeding on a sufficiently large scale. Thus the findings presented here, of the existence in the mouse of transferrin differences very similar to those already found in other mammalian species, may open the way to the experimental detection and analysis of differences in fitness between animals differing in genotype at the transferrin locus.

5. SUMMARY

1. The plasma proteins of six inbred strains of mice have been studied, using starch-gel electrophoresis.

2. The existence of two alternative plasma transferrin (β-globulin) phenotypes has been demonstrated. Five of the strains have one of these and one strain has the other. Each of the two transferrin patterns comprises three (or possibly only two) electrophoretic bands. The two patterns differ in all of these bands.

3. The two transferrin types recognized are determined by a pair of allelic, autosomal genes (designated Trf^A and Trf^B). The TrfA phenotype (CBA strain) is determined by the genotype Trf^A/Trf^A, and the TrfB phenotype (A, C57BL, JU, KL, RIII strains) by the genotype Trf^B/Trf^B. The phenotype TrfAB, of the heterozygote (genotype Trf^A/Trf^B), is distinguishable and shows four (or possibly only three) bands. In this way it closely resembles a mixture of equal parts of TrfA and TrfB plasma.

4. No linkage was detected between the *Trf* locus and sex, the agouti locus or the haemoglobin locus.

5. The possible molecular basis of the action of the transferrin alleles in the mouse, and the widespread distribution in mammals of polymorphism involving the transferrins, are discussed.

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Genetical studies on the skeleton of the mouse

XXVI. PINTAIL*

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INTRODUCTION

The gene for Pintail (symbol *Pt*; linkage group VIII) in the mouse was first described by Hollander & Strong (1951). The original mutant occurred in an experiment in which mice of the 'pBr' strain were treated with the alleged mutagen methylcholanthrene. The animals used in the work described in this paper were descended from four Pintail animals received from the Roscoe B. Jackson Memorial Laboratory, Maine, U.S.A. in August 1956. These animals were initially outcrossed to the CBA/Gr inbred strain, and the present line is descended from these crosses.

GENETICS

According to Hollander & Strong (1951) the mutant showed no dominance. Matings between Pintail heterozygotes produce offspring which can be classified at birth into three phenotypic classes: normal, 'moderate' Pintail and 'extreme' Pintail, the distinction between the two Pintail phenotypes being made on the basis of tail length. 'Extreme' Pintail animals are almost always *Pt*/*Pt*: in backcross ('extreme' Pintail × 'moderate' Pintail) and in intercross matings they are produced in the proportions of a half and a quarter respectively.

There may be a certain amount of mis-classification between Pintail homozygotes and heterozygotes, but all test-matings have confirmed the original classification. The breeding data collected in this laboratory are set out in Table 1. Animals classified as 'moderate' Pintails have been assumed to be genotypically *Pt*/+, and those classified as 'extreme' Pintail to be *Pt*/*Pt*.

Table 1. *Segregation of Pintail*

Type of mating	Normal	*Pt*/+	*Pt*/*Pt*	Total
1. *Pt*/+ ♂ × +/+ ♀	151	125	0	276
2. +/+ ♂ × *Pt*/+ ♀	97	94	0	191
3. *Pt*/+ × *Pt*/+	157	337	148	642
4. *Pt*/*Pt* × +/+	0	46	0	46
5. *Pt*/*Pt* × *Pt*/+	0	42	39	81
6. *Pt*/*Pt* × *Pt*/*Pt*	0	0	117	117

* Work carried out in partial fulfilment of requirements for degree of Ph.D. (London).

Interaction of Pintail and other genes

The effect of Pintail in combination with Brachyury (*T*), Danforth's short-tail (*Sd*) and vestigial-tail (*vt*) was studied. Reciprocal crosses were made between Pintail and these genes. Litters were killed at birth and stained with methylene blue for cartilage. No differences were found between reciprocal matings of the same type.

1. $Pt/+ \times vt/vt$

The offspring were classified as;

22 normals; 15 with *Pt*-type tail.

Hence it was concluded that Pintail and vestigial-tail heterozygotes do not interact.

2. $Pt/+ \times Sd/+$

Animals carrying the *Sd*-gene have an abnormal axis-atlas articulation and hence can be recognized in clearance preparations (Theiler, 1951, Grüneberg, 1953*b*).

The observed segregation was;

24 normals, 21 with *Pt*-type tail, 29 *Sd* animals with a short tail, 14 *Sd* animals with a filamentous tail.

If the last class is genotypically $Sd/+$; $Pt/+$ the segregation is reasonably close to the 1 : 1 : 1 : 1 expected segregation.

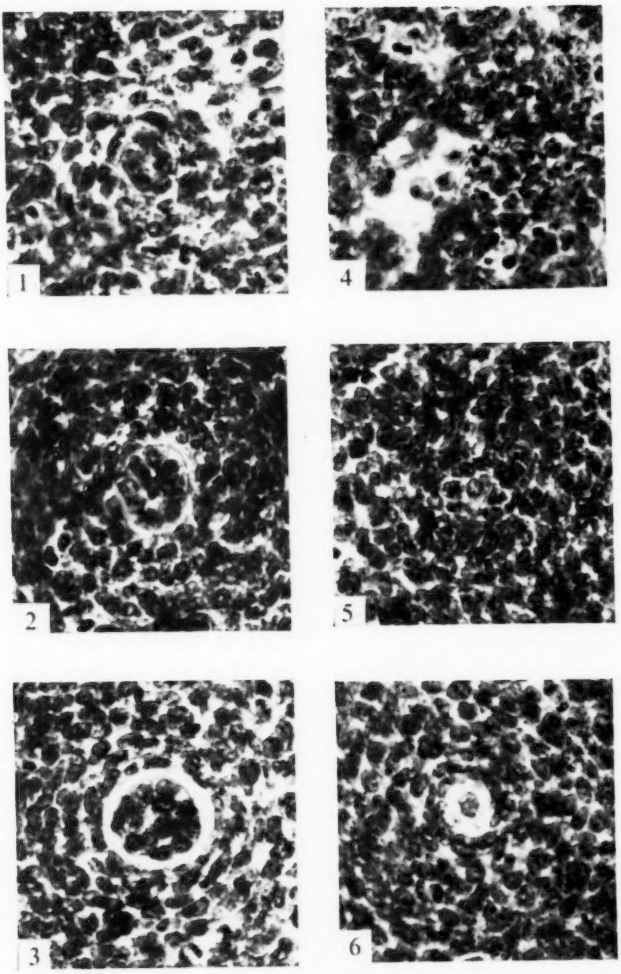
However the *Sd* gene has a relatively strong expression on the genetic background of the *Pt* stock. In a cross between an *Sd* male and a $+/+$ female from the *Pt* stock, the progeny were classified as follows;

16 normal, 22 $Sd/+$ animals.

The *Sd* animals all had very short tails, and five of them had only filamentous tails. Hence it would be incorrect to assume that the fourteen animals with filamentous tails from the $Pt/+ \times Sd/+$ cross are necessarily of $Sd/+$; $Pt/+$ genotype. It is likely that most of them are, since there is no deficiency of *Sd* animals, but the only conclusion that can be made is that there may be some mis-classification of $Sd/+$ and $Sd/+$; $Pt/+$ animals. All the animals with filamentous tails which were not killed at birth died before weaning and thus could not be tested genetically.

3. $Pt/+ \times T/+$

The $Pt/+$ gene on the genetic background of the *T*-stock shows a reduced manifestation. As the effects of the *T* and *Pt* genes on the tail are rather different, it was possible to classify $Pt/+$ animals among the offspring with a reasonable expectation of accuracy. Distinguishing between the other two abnormal genotypes was more difficult. Grüneberg (1958*a*) found that, although early *T* embryos possess a notochord from head to tail-tip, in a single 10½-day-old embryo (which was the



1–6: 7·5 μ transverse sections of the tail of embryos from litter 2 (nominal age $13\frac{1}{3}$ days).

1–3: Respectively 50th, 200th and 400th sections from the tail tip in a normal embryo (C.R.L. 9·9mm.).

4–6: Comparable sections in a *Pt*/+ embryo (C.R.L. 10·1mm.). At the tail tip there is considerable pycnosis and disorganization and no signs of a notochord. The notochord is very small when it eventually becomes visible (6). A trace of the notochord can be seen in 5.

oldest that he examined), 'the notochord was lost track of for a stretch in the cloacal region. . . . It may be that in this embryo the notochord may have actually disintegrated for a distance.' It was found that the nuclei pulposi were reduced in the thoracic and lumbar regions in clearance preparations of some newborn *T*/+ mice. Hence it would appear that there was a secondary breakdown of the notochord in these embryos.

Now the *Pt* gene also reduces the size of the nuclei pulposi (see below) and among the abnormal animals resulting from the *Pt*/+ × *T*/+ cross were a number with a filamentous tail, and these animals had the most abnormal discs. If these animals are taken to be double heterozygotes, the offspring of the cross can be classified as follows

$$32 +/+,\ 24\ Pt/+,\ 28\ T/+,\ 11\ Pt/+;\ T/+$$

As there is no deficiency of *T*/+ animals, and as no filamentous-tailed animals were found among the offspring of the cross between *T*/+ and a normal from the *Pt* stock, this classification is probably substantially correct. Hence the conclusion that *Pt* and *T* reinforce each other.

ANATOMY

The morphology of Pintail mice from birth onwards was described by Hollander & Strong (1951). Generally the tail of Pintail heterozygotes is about two-thirds normal length with a peculiar thin and distorted tip (hence the name) with one or more distal kinks. Alizarin red clearance preparations showed that the kinks are formed by the displacement from correct alignment of normal tail vertebrae. No ankyloses were found. Only the actual vertebrae involved in a 'kink' are abnormal: more distal vertebrae become normal again except at the extreme and of the tail, where the last few vertebrae are small and somewhat irregular. The number of tail vertebrae is usually reduced by about a third, but there were some Pintail animals with the normal number of tail vertebrae. Measurements of the overall length of tail vertebrae revealed no difference between normal and Pintail animals.

Pintail homozygotes differed from heterozygotes only in having a much shorter and more kinked tail, the number of tail vertebrae being always less than ten.

'The Pintail trait is almost as easy to recognize in newborn young as in adults. The tail of the normal newborn mouse has a smooth contour and blunt tip; that of Pintail is more irregular in contour, usually with one or more constrictions, and has a pronounced filiform tip. In the more pronounced cases even half or more of the tail may be thin, and may be bent down and reflexed somewhat like a bent pin' (Hollander & Strong). The classification of all segregating litters was made at birth or soon afterwards, and from the close agreement between the expected and observed segregation ratios, is presumably substantially correct. However, on several occasions it was found that animals classified as Pintail at birth seemed to have a normal tail later in life. Methylene blue clearance preparations (see below) showed that in fact the birth classification was correct and animals which are

clearly Pintail at birth may later become phenotypically normal. In alizarin preparations of up to and including 12-day-old animals no abnormal tails were found. The 15-day-old animals showed the usual pattern of tail deformity. The shortening of the tail with the production of a terminal filament, and the kinking of the tail found in adult mice are almost certainly different manifestations of the gene. The filament falls off the tail as the mouse grows up, and the tail tip may thereafter appear reasonably normal. The tail kinks are probably the incidental result of instability in the axial skeleton so that deforming stress, even that normally incident on a mouse tail in a small cage, might easily result in the displacement of vertebrae. Such instability would be expected because Pintail mice have abnormal intervertebral discs (see below). This explanation accords well with the apparently random distribution of tail-kinks in the distal part of the tail, and the occurrence of normal vertebrae distal to a kink. Hence tail kinks would not be expected to be an inevitable accompaniment of the Pintail trait; the most constant feature would seem to be the form of the tail tip.

Thirty-one *Pt*/+ and twenty-five normal skeletons enzymatically macerated with papain were studied for the occurrence of minor variants (Grüneberg, 1955; Deol and Truslove, 1957). Only two small effects of the Pintail gene (or a gene closely linked to it; see Grüneberg, 1955) were revealed: an increase in the occurence of the interfrontal bone of the skull ($\chi^2 = 5{\cdot}3$, $P = 0{\cdot}02$) and a decrease in the incidence of sacralization of L.VI ($\chi^2 = 3{\cdot}6$, $P = 0{\cdot}06$).

Pintail heterozygotes are not known to have any abnormality other than that of the skeleton. Hollander (1951) described urinogenital abnormalities in one branch of his Pintail stock.

In this line homozygous Pintail animals frequently had a persistent cloaca or an imperforate anus, the latter resulting in early death. Pintail homozygotes of the present stock frequently die during the first fortnight of life. Such animals are invariably retarded in growth and possess a distended belly. On dissection the stomach and intestines are found to be packed with food right down to the anus. One animal which was found dead on the second day after birth was found to have an imperforate anus. However, most of the *Pt*/*Pt* animals that die, do so about the end of the first week and certainly have some anal opening. A possible explanation for the cause of death of these animals seems to be that the size of the anus is insufficient to cope with the faeces, and hence they suffer from a chronic anal blockage.

THE INTERVERTEBRAL DISCS

Some time ago Grüneberg made an incidental observation that the nucleus pulposus of the intervertebral disc was very clearly demonstrated in young mice stained with methylene blue for cartilage and cleared in methyl salicylate (Noback, 1916, see also Grüneberg, 1953*a*). It was found that animals aged about three weeks were the best for this purpose. The animals were killed and the vertebral column removed. The spine was then stretched, tied to a flat glass plate and fixed in formalin. The method of staining and clearing was the same as for whole younger animals.

It was found that the nuclei pulposi were very much reduced throughout the vertebral column in Pintail animals. Drawings of preparations of a normal and a Pintail animal are shown in Fig. 1. It is evident that there are differences in both the size and the shape of the nuclei pulposi between the two animals. The abnormal seems to have more compact and densely staining discs, particularly in

Fig. 1A Fig. 1B

Fig. 1. Drawings of methylene blue stained preparations of mice aged 25 days to show the variation in size and shape of the nuclei pulposi in normal and *Pt/+* animals. The convention of shading is the same throughout, so that although the nuclei pulposi in the tail of the Pintail animal appear larger than in the thoracic region, they in fact contain less notochordal material.

A. Trunk region: sixth cervical vertebrae (at the top) to thirteenth thoracic.
B. Tails of the same mice.

the thoracic region. However, this may be a direct effect of the dorsoventral depth of the discs: the normal animal has densely stained discs in the thoracic region, and flatter and thinner ones in the lumbar region. The only irregularity found in the nuclei pulposi was in the last one or two lumbar discs of Pintail mice in which the nucleus pulposus sometimes seemed to be split in the middle taking the form of two partly overlapping plates. The shape of the nuclei pulposi changes in a

characteristic fashion from head to tail, but the variation seems to be about the same in both Pintails and normals. The ratio of the two major diameters (anterio-posterior and lateral) of the nucleus pulposus is approximately the same in all three genotypes in most regions except the tail, with the exceptions noted above.

It was impossible to see if the nuclei pulposi at a tail kink differed from neighbouring ones, because there seems to be virtually no nucleus pulposus in the region of the tail where kinks occur.

Histological studies on the intervertebral disc

Serial sections were cut of selected intervertebral discs from fixed normal and *Pt*/+ mice. The findings confirmed the results obtained from the study of methylene blue clearance preparations. Discs from *Pt*/+ animals possessed a very much smaller nucleus pulposus than normal, containing fewer cells and already undergoing signs of degeneration at the age of 100 days. The age changes of the discs in Pintail mice will be the subject of a separate communication.

EMBRYOLOGY

Hollander & Strong (1951) state that recognition of the Pintail trait is 'possible in late foetal stages', but attempted no further embryological analysis. In the present study embryos were collected from both backcross (Pintail × normal) and intercross (Pintail × Pintail) matings. The chronological age of the embryos was known to within one night, and this time was checked by measuring the crown-rump length of the embryos, and comparing it with the standard series given by Grüneberg (1943). Twenty-one litters containing 130 embryos ranging in size (average C.R.L.) from 11·6 mm. (14½ days old) to 2·4 mm. (9 days old) were serially sectioned. A camera-lucida drawing was made of every embryo before it was sectioned. The material was fixed in Bouin's fluid and embedded by Peterfi's collodion impregnation method (Peterfi, 1921). Sections were cut at 7·5 μ or 8 μ except in the case of the large embryos of litter 3 and the trunk region of litter 1 which were sectioned at 10·0 μ. The sections were stained with Ehrlich's haematoxylin and eosin. Great care was taken to obtain, as nearly as possible, transverse sections through the tail, the tail bud or the posterior end of the body, as interpretation of these regions is difficult or even impossible in oblique sections (Grüneberg, 1956). In the larger embryos (litters 1–2, 11–12) the tail was cut from the trunk and sectioned separately. The trunk region was then available for sectioning in a more favourable plane. Projection drawings at magnification × 250 were made of every tenth section of the tail region of each embryo.

The serially sectioned embryos are listed in Table 2. About half a dozen embryos are omitted from the classified litters because of mechanical damage during preparation which made classification impossible. Normals and abnormals could be recognized by external inspection in litters 1–2, 11–12; as classified from the sectioned material, litters 3–7 contained 12 +/+ and 15 *Pt*/+ embryos, and litters 11–17 8 +/+, 16 *Pt*/+ and 12 *Pt*/*Pt* embryos. There was no detectable effect

Table 2. *Serially sectioned embryos*

Pt/+ × +/+ matings					*Pt*/+ × *Pt*/+ matings					
	Nominal age	Mean C.R.L.	No of embryos			Nominal age	Mean C.R.L.	No. of embryos		
Litters	(days)	(mm.)	+/+	*Pt*/+	Litters	(days)	(mm.)	+/+	*Pt*/+	*Pt*/*Pt*
1	14½	11·5	5	3	11	14½	11·6	1	2	2
2	13½	10·0	1	6	12	12½	8·0	1	2	2
3	12	7·7	3	2	13	11	5·4	1	1	2
4	11½	6·3	2	2	14	10½	4·5	1	2	2
5	11½	6·1	3	4	15	10½	4·3	3	1	1
6	10½	4·5	2	4	16	10	3·7	1	4	2
7	10	3·7	2	3	17	10	3·7	0	4	1
9	9½	2·8	6*							
10	9	2·4	5*							

* Not classified.

of the genotype upon size (crown rump length), and therefore only the mean size of litters are given in the table.

The Development of Pt/+ *and* Pt/Pt *Embryos*

14½-day-old embryos. At the age of 14½ days, the tail tip of Pintail embryos appears constricted and tapering. It was not found possible to distinguish between *Pt*/+ and *Pt*/*Pt* embryos by external inspection at this age. Sections of the tail of *Pt*/+ animals of litter 1 showed some pycnotic cells in the tail tip and considerable terminal disorganization. The notochord was very much reduced and terminated some way before the actual end of the tail. There was no proper notochord terminally although in places the remains of the sheath with one or two cells, often pycnotic, could be seen. The notochord proper begins about half-way along the tail and is clear and continuous for the rest of its course anteriorly. The cells, which are arranged concentrically around the notochord in the normal, do not show the same degree of orientation in the abnormal, as if some organizing influence which normally emanates from the notochord is absent or reduced. In litter 11 (which is of the same age) there was one normal embryo, two embryos similar to the abnormals in litter 1 and presumed Pintail heterozygotes, and two more severely affected animals. These are presumably Pintail homozygotes. In these two animals there was no trace of the notochord in the tail at all, and pycnosis was much more advanced than in the *Pt*/+ embryos. Distally pycnotic cells were found in all parts of the sections of the tail; more anteriorly pycnosis seemed to be confined more to the central region as if it were radiating from the place where the notochord normally would be.

13½-day-old embryos. The embryos in litter 2, one day younger, were similar in appearance and tail structure to those of litter 1. The appearance of the notochord in sections of two embryos of this age are shown in Plate I. The trunks of two embryos from this litter, one normal, one *Pt*/+, were serially sectioned so that the notochord was cut transversely over as great a region as possible. Counts were

made of the number of cells in the notochord (Fig. 2). Abercrombie (1946) has drawn attention to the possible inaccuracies involved in the making of cell counts from microtome sections. No attempt has been made to apply this correction as all counts were made for comparison with a similar series. The number of cells in the *Pt*/+ embryo is reduced by about a third.

The only difference between the two animals seems to be in the reduced number of cells in the *Pt*/+ animal in the regions of cell aggregation. These are the cells which persist until birth and eventually become the nucleus pulposus of the intervertebral disc.

12½-*day-old embryos*. Litters 3 and 12 differed from the older ones only in showing less pycnosis. At this stage Pintail embryos cannot be recognized with certainty from their external appearance, and hence the classification had to be made entirely from sections. Although the notochordal sheath had been formed in the

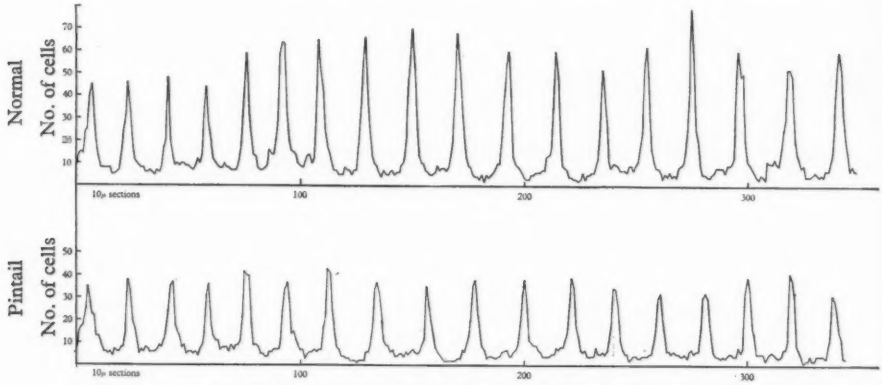


Fig. 2. Number of cells in the notochord of the trunk region of a normal and a *Pt*/+ embryo of litter 2 (nominal age 13⅓ days, C.R.L. 9·9 mm. and 10·1 mm. respectively), showing the progressive reduction in number of the cells in the aggregations in the Pintail embryo along the axis of the embryo.

trunk region, it had not extended into the tail of these embryos. This made the interpretation of the tail sections more difficult and although in many places it was impossible to recognize a notochord, it was possible that a small one was present.

11–11½-*day-old embryos*. At this stage condensation of mesenchyme round the notochord of the tail has barely begun, and hence the notochord is clear in section. There seems to be no marked pycnosis in the sectioned embryos examined (litters 4, 5 and 13). It was possible, at least in litters 4 and 5, to recognize the abnormal animals by a rather smaller tail notochord, but the difference in size was not striking. Although measurements of the cross-sectional area and counts of the number of notochordal cells tended to confirm the classification made by eye, convincing differences between the three genotypes were, at this stage, only revealed by mitotic counts (see below).

9–11-*day-old embryos*. It was not found possible to recognize Pintail embryos at ages younger than 11 days from a cursory inspection of sectioned material. All

embryos have an apparently normal tail both in overall size and in the size of the notochord. Now Pintail embryos of 11 days and older have a reduced notochord which leads to a breakdown of the tail tip. The reduction of the notochord is not yet obvious in 10-day embryos. It may have been brought about by:

i. the death of cells
ii. the failure of cells to increase in number
iii. the loss of notochordal cells by migration and incorporation in other tissues.

To test these alternatives the number of cells, pycnoses and mitoses in the notochord were counted in a region immediately in front of the tail tip (sections 30–50 from the tip) in a total of 55 embryos (data summarized in Table 3: the mitotic rate counts fall into distinct groups which have been pooled). In the two youngest litters 9 and 10 it was found impracticable to count the cells in the notochord in

Table 3. *Mitotic rates (mitosis/cell) in the notochord of normal and abnormal embryos (sections 30–50 from the tail tip). The upper section of the table from* Pt/+ × +/+ *matings, the middle section from* Pt/+ × Pt/+ *matings. In the lower section, comparable figures are given from some* +/+ *embryos from Grüneberg's* (1958*b*) Sd *experiments, prepared by the present author. Individual counts, in all but five cases, are based on 200–500 cells. Numbers of embryos in parentheses.*

Litters	Mean C.R.L. (mm.)	Mitotic rate +/+	*Pt*/+	*Pt*/*Pt*
5	6·1	0·118 (3)	0·065 (4)	—
6	4·5	0·194 (2)	0·098 (4)	—
7	3·7	0·194 (2)	0·119 (3)	—
13	5·4	0·129 (1)	0·096 (1)	0·054 (2)
14	4·5	0·202 (1)	0·105 (2)	0·070 (2)
15	4·3	0·200 (3)	0·114 (1)	0·086 (1)
16	3·7	0·257 (1)	0·138 (4)	0·079 (2)
17	3·7	—	0·129 (4)	0·076 (1)
	5·1	0·177 (2)		
	4·4	0·199 (5)		
	3·7	0·275 (3)		

In addition to the litters tabulated above, two younger litters from *Pt*/+ × +/+ matings have been examined in which normal and abnormal embryos could not be distinguished. One, of six embryos (litter 9, mean C.R.L. 2·8mm.) had a mean mitotic rate of 0·483 mitoses/cell; the other, of five embryos (litter 10, mean C.R.L. 2·4mm.) had a mean mitotic rate of 0·401.

section 30 because at this age the notochord has not yet become differentiated from its neighbours in this region and in these two litters the counts were made on sections 35–50 inclusive in front of the tail tip. The mitotic and pycnotic rates are not affected by the plane of sectioning (unlike cell number) and hence provide a reasonably accurate estimate of the rate of increase or decrease in cell number. A cell was scored as being in mitosis if its chromosomes were clearly visible.

No embryos had more than a few pycnotic cells in the notochord, but there were

marked differences in mitotic rates between different embryos. Fig. 3 includes counts from both backcross (indicated by ○) and intercross (indicated by ●) matings. Also included (indicated by ×) are data from normal embryos of another stock (see Table 3).

Embryos from intercross matings (litters 13–17, Table 2) are distributed in

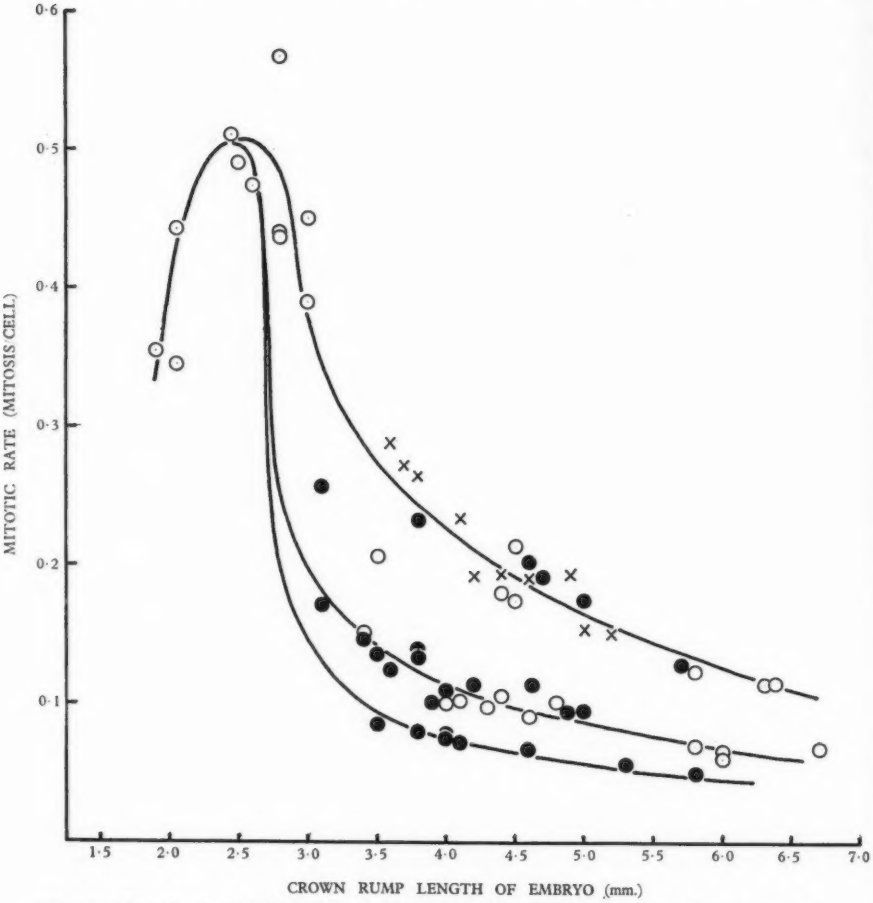


Fig. 3. Mitotic rate in the embryonic tail notochord showing the different rates of cell division in +/+, *Pt*/+ and *Pt*/*Pt* embryos. The rate in embryos from backcross matings is indicated by ○, from intercross matings by ●. The top line represents the mitotic rate in normal embryos, the bottom line in putative *Pt*/*Pt* embryos. Normal embryos from Grüneberg (1958*b*) are indicated by ×.

three distinct groups in the figure in the proportions 6 : 12 : 8. Embryos from litters 5–7 (from backcross matings) fall into the two upper groups in Fig. 3, in the proportion of 7 : 11. Hence it seems reasonable to conclude that the mitotic rate in the notochord of normal mice is shown by the top line of Fig. 3, that the second line includes the *Pt*/+ embryos, and the bottom line the *Pt*/*Pt* embryos.

Moreover litter 5 can be classified directly from the appearance of the notochord in sections. This classification is the same as the one made on the basis of mitotic counts.

Fig. 3 shows that in normal embryos of 3·5 mm. C.R.L. or over the mitotic rate of the cells of the notochord decreases steadily. This process is accelerated mildly in *Pt*/+ and strongly in *Pt*/*Pt* embryos. The reason for this difference is not clear. It is possible that the separation of the notochord from tail gut and neural tube happens in a rather different way in Pintail embryos, but the available data are not adequate to establish this point and will not be discussed.

Litters 9 and 10 contain 11 embryos. The probability of any embryo being genotypically *Pt*/+ is 0·5. Hence the probability of there being no Pintail animal in this group is $(\frac{1}{2})^{11}$, which can clearly be neglected. However it is clear from Fig. 3 that Pintail embryos cannot be identified before the age of 10 days on the basis of the mitotic rate in the notochord.

The average mitotic rates in the notochord of the tail tip of embryos collected by Grüneberg (1958*b*) are also given in Table 3. It is clear that the mitotic rates in the notochord of these embryos and those in the Pintail stock inferred to be normal are very similar. The mitotic rate in the known normal embryos is slightly higher than in the embryos from the Pintail stock. This may just be experimental error, but it may represent a true difference in growth rate between the notochords of the two series of embryos. The known normal series of embryos is from the *Sd*-stock, and this is known to have different modifying genes to those present in the *Pt*-stock (see above).

It may be mentioned here that the tail gut and the ventral ectodermal ridge of the tail (Grüneberg, 1956) are of approximately normal size.

CONCLUSIONS ABOUT THE EFFECT OF THE *Pt* GENE ON DEVELOPMENT

From the data presented in the previous sections it is possible to arrive at a fairly complete understanding of the causal relationships in the effect of the *Pt* gene on development. The findings can be most conveniently summarized in a 'pedigree of causes' of the gene effects (Fig. 4). The accelerated age changes of the nuclei pulposi of the intervertebral discs will be discussed in a separate communication (Berry, 1960).

SUMMARY

1. The gene for Pintail (symbol *Pt*) shows no dominance. Heterozygotes are viable and fertile; many homozygotes die early in life, but the survivors appear to breed normally. *Pt* reinforces the effects of *Sd* and *T* on the tail; there is no evidence for interaction between *Pt* and +/*vt*.

2. The gene produces a shortening of the tail, but the most marked effect on the adult is in the great reduction of the nucleus pulposus of the intervertebral discs, which may lead to tail kinks in some animals.

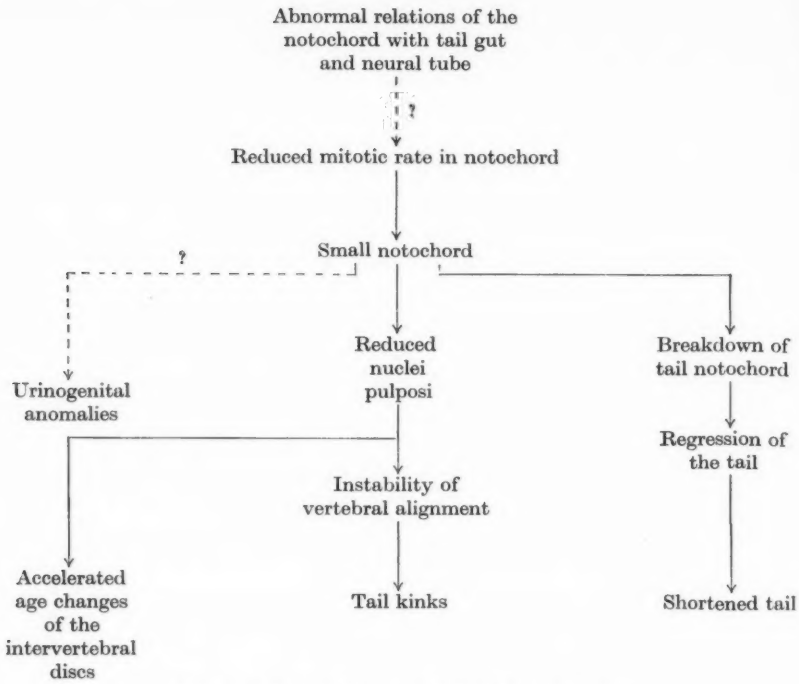


Fig. 4. Pedigree of causes of the *Pt* gene effects.

3. Pintail embryos can be recognized from the eleventh day stage of development onwards by the possession of a much smaller notochord than normal. This reduced notochord is the result of a reduced rate of cell division which first manifests itself in 10-day embryos. Perhaps this reduced rate is the result of altered relations of the notochord with tail-gut and neural tube in 10-day embryos.

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Comparative mutability of wild-type isoalleles at the white loci in *Drosophila melanogaster*

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1. INTRODUCTION

Timoféeff-Ressovsky (1932) reported that two phenotypically indistinguishable wild-type stocks of *Drosophila melanogaster*—designated American and Russian—possessed distinguishable wild-type alleles at their white eye colour (*w*) loci. The w^+ alleles of two stocks were found to mutate at significantly different rates following X-irradiation of adult males. Subsequently, Muller (1935) showed that Timoféeff's Russian and American w^+ alleles could be phenotypically separated by virtue of the different phenotypes of triploid females carrying two doses of a *w* mutant and one or the other w^+ allele. As a result of their demonstration of different wild-type alleles at the *c.i.* locus in *D. melanogaster*, Stern & Schaeffer (1943) designated such alleles, which are difficult to distinguish by conventional genetic methods, as isoalleles. Lefevre (1955) has submitted evidence for the occurrence of mutational isoalleles at the yellow (*y*) body colour locus in *D. melanogaster*.

With the demonstration of the pseudoallelic nature of the *w* locus of *D. melanogaster* (Lewis, 1952; Mackendrick & Pontecorvo, 1952; Green, 1959*a*)—with four recombinationally discrete loci indicated—a re-examination of the genetic basis of isoallelism at these loci was undertaken. Evidence has been presented demonstrating that at least two distinctive w^+ isoalleles occur among a number of laboratory-maintained wild-type stocks (Green, 1959*b* and unpublished). Furthermore, it was shown that the isoalleles are localized to the two right members of the four w^+ loci (Green, 1959*b* and unpublished).

These observations demonstrate that Timoféeff's Russian and American stocks are not unique and motivated a restudy of the comparative mutability of the w^+ isoalleles. For a number of reasons such a restudy is indicated. First, with the detailed genetic analysis of the *w* loci now available, induced mutants can be localized to specific *w* loci. Second, since Timoféeff's study, much data on the frequency and kind of spontaneous and X-ray-induced *w* mutants has been accumulated. In both frequency and eye-colour phenotypes of the *w* mutants these data are at variance with those of Timoféeff. These facts will be discussed *in extenso* below. Therefore, experiments designed to obtain answers to these two questions were carried out. What is the comparative X-ray mutability of the two w^+ isoalleles and what is the frequency of mutation among the recombinationally discrete w^+ loci? In brief, the answers to both questions depend upon first inducing a large number of independent mutants, and second determining the recombination relationships of these mutants. While the former task presents no special difficulties, the latter does.

Any analysis of the recombination relationships of a comparatively large group of independently occurring pseudoallelic mutants is, in *Drosophila*, an exceedingly laborious undertaking. Obviously a simpler method of localization is called for. In the case of the *w* loci one is available which permits at least a partial linkage assignment. This is a phenotypic method for the specification of linkage position of *w* mutants and stems from observations first made by Gans (1953) that *w* mutants can be classified into two types on the basis of their interactions with the independent eye-colour mutant zeste (*z*). Some *w* mutants act as dominant suppressors of *z*; others do not. An extension and a more detailed analysis of this observation was made (Green, 1959*a*) wherein the recombination relationships and *z* interactions of thirty-six independent *w* mutants were studied. It was found that, without exception, all mutants suppressing *z* are located in the two right *w* loci, whereas those failing to suppress *z* are located at the two left *w* loci. This invariable correlation is taken to mean that suppression can be used with confidence in localizing *w* mutants to either the left or right pairs of *w* loci, thereby obviating the need for the more time-consuming recombination study. For purposes of this study, a partial linkage assignment suffices, and the *z* interaction was so employed.

2. EXPERIMENTAL

The two wild-type stocks chosen for study are the well-known laboratory stocks Canton-S and Oregon-R which have been shown to possess dissimilar w^+ isoalleles (Green, 1959*b*). Canton and Oregon can be distinguished phenotypically from each other by their separable eye phenotypes when compounded with two *w* mutants in triploid females or when heterozygous for a *w* mutant in diploid females (Muller, 1935; Green, 1959*b*). A genetical analysis of these stocks has shown that, of their four w^+ loci, the phenotypic difference is determined by different wild-type isoalleles localized to the two right w^+ loci (Green, 1959*b*). For simplicity the four w^+ loci of Canton will be designated $+^C+^C$ and of Oregon $+^O+^O$, where the left + represents the two left loci, the right + the two right loci. Four stocks were used in this study: (1) $y^2\,z\,+^C+^C$, (2) $y^2\,z\,+^O+^O$, (3) *sc* $z\,+^C+^O$, and (4) *sc* $z\,+^O+^C$. Stocks (1) and (2) represent Canton and Oregon into which the identical markers y^2 (body colour) and *z* were introduced, while (3) and (4) are composite wild-type stocks into which *sc* (scute bristles) and *z* were introduced and containing w^+ loci half each from Canton and Oregon. The notation $+^C+^O$ means the left w^+ loci came from Canton, the right from Oregon; $+^O+^C$ the converse. The synthesis of the composite wild-type stocks has been described in detail (Green, 1959*b*).

Males $y^2z+^C+^C$ and $y^2z+^O+^O$ were irradiated simultaneously with 5000 r X-rays (90 kV, 3 mA) delivered at a rate of approximately 300 r/minute. X-ray output was checked with each irradiation with a Victoreen dosimeter and was found to vary about 10%. Following irradiation, males were crossed to females homozygous for the recessive sex-linked mutants y^2, w^a (white-apricot), *v* (vermilion eye colour) and *f* (forked bristles) contained within the sc^8 and *dl-49* inversions. Ten males were crossed to 15–20 females in half-pint milk bottles containing the

standard corn meal–molasses–brewer's yeast–agar medium. After 4 days, all individuals were transferred to fresh media for a second 4-day period, followed by a second transfer for a third 4-day oviposition period, after which the flies were removed and discarded. F_1 females were scored and all individuals showing altered eye colour and bristle phenotypes were progeny tested by crossing to male sibs. Where *sc z* $+^{c}+^{o}$ and *sc z* $+^{o}+^{c}$ males were used, the identical procedure was followed and body-colour mutations were sought as well.

Depending on the outcome of the progeny tests, the presumptive *w* mutants were handled in one of two ways. Where both y^2 w^a *v f* and *w* mutant males carrying either y^2 *z* or *sc z* were found among the F_2 progeny, the latter were crossed to *y ac z ec* females to determine whether the new *w* mutant did or did not suppress *z*. Where only y^2 w^a *v f* males occurred among the F_2, it was concluded that the chromosome carrying the *w* mutant was male lethal. Now male lethality can be due either to the induction of a *w* lethal mutant or deficiency, or in a few cases to the simultaneous occurrence of a viable *w* mutant and a separate, linked lethal mutation. A simple test was used to distinguish between these two possibilities. It has been previously shown (Green, 1959*c*) that *w* mutants and *w* deficiencies when compounded with the *w* mutant spotted-white (*sp-w*) give phenotypically separable individuals. Thus females *w* mutant/*sp-w* produce a complementary eye colour while females *w* deficiency/*sp-w* have a *sp-w* eye phenotype. Therefore each *w* lethal was compounded with *sp-w*, and if the resultant phenotype was *sp-w*, it was concluded that the *w* lethal was localized to the *w* loci, probably as a deficiency. If, on the other hand, a complementary eye phenotype was found, it was assumed that the lethal chromosome carried a *w* mutant plus a linked, independent recessive lethal and required further testing. Each of these male lethal chromosomes was compounded with a wild-type X chromosome and separation of the *w* mutant and linked lethal through crossing over was sought. Such a separation should result in the recovery of *w*-eyed males. A total of twenty-four independent *w* lethals were found in these experiments. Four gave complementary eye phenotypes on testing to *sp-w*. Subsequently, male viable *w* mutants were recovered from each, demonstrating that the lethal was separable from the *w* loci.

Before presenting the experimental results it is germane to consider the question of the nature of mutations at the *w* loci. On the basis of much experience over the last three decades, it is possible to list the following phenotypic effects which are associated with genetic changes at the *w* loci: (1) viable homogeneous eye colour mutations, (2) lethal *w* mutations and (3) mottled or variegated eye-colour mutations. Each of these mutational events is accompanied by genetic changes which are more or less amenable to cytogenetic analysis. Among the viable, homogeneous eye-colour mutants—especially those apparently induced by X-rays—the following types have been described. The bulk of the mutants are 'point' mutational changes, where 'point' mutation means that such mutants freely recombine with their pseudoalleles. A small proportion of these mutants have been associated with gross chromosomal rearrangements. Thus Demerec (in Bridges & Brehme, 1944) reports that among eight X-ray-induced *w* mutants, seven were cytologically normal and

one was associated with a readily detectable X chromosome inversion. Using crossing over between the mutants *sc* and *z* as a criterion, Green (unpublished) found two of fifteen X-ray *w* mutants to be associated with a chromosome rearragement. All the remainder recombined in an orthodox manner with their pseudoalleles just as did *w* mutants of spontaneous origin. While none has yet been demonstrated, it is conceivable that viable *w* mutants associated with a minute chromosome loss, as described by Green (1959*c*) for certain recombinant exceptions at the *w* loci, could result from X-ray treatment. Such losses, although cytologically not discernable, could be identified by their failure to recombine freely with pseudoalleles.

Among the lethal *w* mutants, two classes of mutants have been described. Some lethals are apparently devoid of a detectable chromosomal alteration while most others are inseparably associated with a chromosomal loss varying from the barely detectable cytologically to gross losses or rearrangements (Valencia & Muller, 1948; Demerec, *vide supra*). The *w* mottled or variegated mutations are, without exception, associated with chromosomal rearrangement in which the w^+ loci are brought into juxtaposition with heterochromatin. It is of relevance here to mention the *sp-w* mutants which genetically behave as 'point' mutations localized to the *w* loci. Because of their phenotype, *sp-w* mutants could conceivably be mistaken for a *w*-mottle if scored solely in males.

On the basis of the available information it is concluded that the great majority of X-ray-induced mutants at the *w* loci are 'point' mutations as defined above.

3. RESULTS

In Table 1 the frequency of *w* mutations recovered among the several irradiation experiments has been compiled. For each irradiated genotype three classes of *w* mutants are listed: *w* mutants, those with a white eye colour; w^x mutants, those with some eye pigment; and *w* lethals, those which are male lethal as described above. In addition, for purposes of comparison, there are included in Table 1 Timoféeff's experiments with the American ($+^A$) and Russian ($+^R$) wild-type isoalleles together with experiments of other investigators who studied the X-ray induction of mutation at *w* loci.

Perusal of the results shows that no difference was found in the overall frequency of *w* mutations induced in the $+^C$ or $+^O$ w^+ loci. These results are therefore strikingly different from those reported by Timoféeff for $+^A$ and $+^R$, where an appreciable difference in rates will be noted. Furthermore, the results reported here for $+^C$ and $+^O$ differ from those of Timoféeff in two more ways. First, the overall mutation rate —considering only male viable mutants—is *ca.* 1/6400 chromosomes in contrast to Timoféeff's report for $+^A$ of 1/1100 and for $+^R$ of 1/1900. The results reported here compare favourably with those of Valencia & Muller (1948), who found for a dose of 5000 r that viable *w* mutants occurred at a frequency of *ca.* 1/8000 chromosomes. Heptner & Demidova (1936) and Glembozky (1936) found essentially identical mutation rates of 1/4000. Lefevre's results are *ca.* 1/6000 while in two separate earlier experiments of the author, rates of *ca.* 1/8000 and 1/6500 were found. As

Table 1. *Comparative frequency of X-ray-induced* w *mutations*

Genotype irradiated ♂♂	Dose, r	Mutations to *w*				Total chromosomes	Viable mutants/ 5000 r/10^5 chromosomes	Authority
		w	w^x	♂ lethal	untested			
$y^2\ z\ +^O+^O$		11	1	3	2	77,905	15·4	
$y^2\ z\ +^C+^C$	5000	11	2	3	0	77,008	16·9	This study
$sc\ z\ +^C+^O$		9	1	5	2	61,603	16·2	
$sc\ z\ +^O+^C$		8	2	9	3	72,115	13·9	
Total		39	6	20	7	288,631	15·6	
$+^C+^C$	5000	11		10	10	72,556	15·2	M. M. Green (unpublished)
$v\ f\ ^{3n}\ car$	5000	14		10	1	132,624	10·6	M. M. Green (unpublished)
$v\ f$*	5000	37		31	51	219,850	16·8	G. Lefevre (unpublished)
$+^A$	4800	41	14	—		59,200	92·9	Timoféeff (1932)
$+^R$	4800	19	21	—		75,300	55·1	Timoféeff (1932)
±(?)	4800	25	12	—		48,500	76·3	Timoféeff (1933)
±(?)	5000–6000	43	10	—		106,500	49·8	Timoféeff (1939)
wild-type	4000	20 (25)†	3 (4)	7	22	106,674	27·2	Heptner & Demidova (1936)
wild-type	6000	8 (7)	2	7	18	46,800	19·2	Heptner & Demidova (1936)
wild-type	4000	21 (26)	3 (4)	8	22	109,803	27·3	Glembozky (1936)

* Summation of results where three different *v* and *f* alleles were used.
† Numbers in ( ) are computed for 5000 r.

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indicated in Table 1, Timoféeff consistently obtained comparatively high *w* mutation rates. His 1933 results were *ca.* 1/1300 chromosomes, and those of 1939, 1/2000 chromosomes.

It should be noted that presumptive *w* mutants recorded as untested females in Table 1 have not been included in the computation of mutation rates. The proper disposition of these individuals presents a number of difficulties. In one approach it may be assumed that the *w* mutants of untested females are distributed proportionally among the male viable and lethal classes. By such an apportionment higher rates accrue for all the experiments tabulated other than those of Timoféeff. There is good reason to believe that such an apportionment is neither fair nor realistic. Experience shows that many presumptive mutants detected in females turn out, on progeny testing, to be mosaics arising through chromosome elimination. Any group of untested mutants must be corrected for these mosaics, a correction which undoubtedly varies from experiment to experiment.

The data presented here, together with those of Heptner & Demidova (1936) and Glembozky (1936), differ from those of Timoféeff in yet another respect. This is the ratio of pure white-eyed mutants to pigmented mutants. With the exception of his 1939 study, Timoféeff reports the occurrence of many more pigmented *w* mutants than other investigators listed in Table 1. Moreover, the finding that the $+^R$ isoallele mutates as frequently to pigmented mutants as to white is not in keeping with the results listed here for either $+^C$ or $+^O$, nor with the experience of other investigators. It is of interest to note that among the six pigmented mutants listed in Table 1 derived from $+^O$ and $+^C$, five ranged from tinged to apricot-like, one is a spotted-white allele.

Only brief mention need be made at this point of the *z* reaction of the mutants recovered here. The results, listed in Table 2, show that the bulk of the mutants fail

Table 2. *The* zeste *reaction of X-ray-induced* w *mutants*

Source of *w* mutants	Number of *zeste*	
	Suppressors	Non-suppressors
$y^2\ z\ +^O+^O$	2	10
$y^2\ z\ +^C+^C$	2	11
$sc\ z\ +^C+^O$	2	8
$sc\ z\ +^O+^C$	3	7
Total	9	36

to suppress *z*. The ratio of *z* suppressors to non-suppressors is 1:4. These data parallel those reported for spontaneous *w* mutants where, among 24 chosen at random, 6 were *z* suppressors, 18 not (Green, 1959*a*). By using suppression of *z* as a basis it is possible to compare the mutational frequencies of the left and right halves of the $+^C$ and $+^O$ stocks. From the data of Table 2 it can be readily seen that these rates are essentially identical. A total of 4 mutants/149,123 chromosomes (*z* suppressors) were localized to the right $+^C$ loci, 18/138,611 (non-suppressors of *z*) to the left $+^C$

loci. For the $+^{O}$ loci the respective totals are 5/139,508 for the right loci and 18/150,020 for the left loci.

Since mutations at the *y*, *v* and *f* loci were also recovered, their frequency is given in Table 3. In the case of the lethal mutants at these loci no attempt was made to determine the frequency of those which were, in fact, a viable mutant plus an independent lethal simultaneously induced. Judging from the *w* mutation data and the X-ray dose used, about 15% of the lethal mutants belong to this category. Thus the viable mutant frequencies are almost certainly slightly low.

Table 3. *Comparative frequency of induced mutations at* y, v *and* f *loci*

Genotype irradiated ♂♂	Mutations to *y*			Mutations to *v*			Mutations to *f*			Total chromosomes
	♂ viable	♂ lethal	untested	♂ viable	♂ lethal	untested	♂ viable	♂ lethal	untested	
$y^2 z +^{O}+^{O}$				5	5	29	9	9	3	77,905
$y^2 z +^{C}+^{C}$				4	10	36	3	12	2	77,008
$sc\, z +^{C}+^{O}$	2	0	0	5	8	39	6	8	2	61,603
$sc\, z +^{O}+^{C}$	5	4	2	8	9	34	7	5	0	72,115
Total	7 (5·2)*	4	2	22 (7·6)*	32	138	25 (8·7)*	34	7	288,631

* Viable mutants/5000 r/10^5 chromosomes.

A few comments on these data are warranted. The frequency of *f* mutants found is compatible with the report of Valencia & Muller (1948), who state that for 5000 r the *f* mutation rate is *ca.* 1/8000 treated chromosomes. Other investigators report somewhat higher rates as follows: both Heptner & Demidova (1936) and Glembozky (1936) *ca.* 1/6000; Timoféeff (1939) *ca.* 1/4000. Among the *f* lethal mutants found, one-half were associated with a concomitant Minute bristle effect suggesting that these lethals are probably small deletions. The frequency of *v* mutants listed here is appreciably greater than the 1/22,000 frequency noted by Valencia & Muller (loc. cit.) for an equivalent X-ray dose. It will be noted that a striking frequency of F_1 *v* females was found in these experiments, most of whom were sterile on progeny testing. No explanation for this high incidence is immediately obvious.

4. DISCUSSION

The first conclusion warranted by these experiments is that, taken as a whole, the w^+ loci of the Canton and Oregon wild-type stocks mutate at essentially identical rates following X-irradiation. Further, the data show that the two right w^+ loci of Canton and Oregon—demonstrably different (Green, 1959*b*)—mutate at identical rates. Finally, their left w^+ loci which thus far have not been phenotypically separated also are mutationally inseparable. The overall *w* mutation rates both as to frequency and phenotypes of mutants reported here are wholly at variance with those reported by Timoféeff (1932) for the $+^{A}$ and $+^{R}$ wild-type stocks. While it is not possible to reconcile completely the sets of contradictory data, some facts merit discussion. It is, of course, possible to explain away the differences by invoking the

argument that since the wild-type stocks used here were not identical to those of Timoféeff, there is no basis for discussion. Such an argument is insufficient since there is good reason to believe that the w^+ loci of Timoféeff's $+^R$ and $+^A$ stocks are not totally different from those of Canton and Oregon. Precisely those phenotypic criteria which Muller (1935) employed to distinguish the w^+ isoalleles of $+^R$ and $+^A$ were adopted for separating the w^+ isoalleles of $+^C$ and $+^O$ (Green, 1959*b*). On the basis of these criteria, $+^R$ is equivalent to $+^C$, and $+^A$ to $+^O$. While it is recognized that phenotypic criteria alone cannot establish identity among the w^+ isoalleles, they do suggest in the very least that the wild-type stocks studied by Timoféeff are not widely different from those used here. In addition, a study of eight independent wild-type stocks shows that they fall into but two classes insofar as phenotypic criteria allow the identification of the w^+ loci: six are inseparable from Canton, two from Oregon (Green, unpublished). A detailed genetic analysis of two (Crimea and Formosa) shows that they differ at their two right w^+ loci just as do Canton and Oregon. From these considerations the significant differences in mutation rates of Timoféeff's two stocks and the identical rates of the $+^C$ and $+^O$ stocks are difficult to explain. Needless to say, the situation is confounded by the fact that nothing specific can be stated concerning the extensive genotypic difference which might exist among these wild-type stocks at other loci, loci which could significantly influence the mutation rates of the w^+ loci. However, Timoféeff (1932) presented data to show that the mutation rates of $+^R$ and $+^A$ were quite independent of their background genotypes. Conceivably the background genotypes of $+^R$ and $+^A$ could be sufficiently different from that of Canton and Oregon to bring about the differences noted. In this connexion it should be noted that the mutation rates reported here are fully compatible with rates found by other investigators using, in all likelihood, wild-type stocks of diverse origin. While these facts suggest that the different findings of Timoféeff's and here are not wholly inherent in the nature of the wild-type stocks employed, they, unfortunately, do not provide a clear-cut basis for explaining the differences.

Another difference, similarly difficult to explain, is the comparatively high frequency of pigmented *w* mutants observed by Timoféeff and not correspondingly by others. Perhaps this difference can be explained, in part, by the mutation methods employed. Throughout Timoféeff's experiments, males were irradiated, crossed to attached-X females, and sex-linked mutants sought among the F_1 male progeny. In addition to *w* mutants, eye-colour mutants at other X-chromosome loci will be found among the F_1 males. Since some of these non-allelic loci, e.g. garnet and ruby eye colours, mutate at rates equivalent to or greater than *w* (Valencia & Muller, 1948), experimental provisions must be made to distinguish among the several mutating loci; and this may often have been impossible because of death or sterility of mutant males. Unfortunately, no information is given by Timoféeff as to precisely how this contingency was handled. Is it possible that other eye-colour mutants were inadvertently classified as *w* mutants? Other workers invariably recovered *w* mutants in F_1 females, thereby effectively eliminating any bias from recessive sex-linked mutants at other loci.

Recent information on the genetical organization of the *w* loci has a bearing on yet another mutational event described by Timoféeff. He (1933) reports that, following X-irradiation, the mutants w^a and w^{bl} separately mutated to w^e. If by w^e is meant the mutant whose phenotype does not manifest dosage compensation, there is good reason to be dubious of this transmutation.

The facts as now known are these. Both w^a and w^{bl} occupy loci distinctive from those of all dosage non-compensating mutants including w^e. Furthermore, w^a or w^{bl} coupled to the same chromosome with w^e produces a near-white eye phenotype. Therefore the transmutation of w^a or w^{bl} to w^e requires the simultaneous occurrence of two events: the reversal of w^a or w^{bl} and the mutation of w^e. Such coincidences must be rare indeed, and the occurrence of two exceedingly rare.

The second conclusion warranted by the mutation data presented here is that the two halves of the *w* loci mutate at significantly different rates. Based upon the *z* suppression test the comparative frequency of mutations in the left and right halves of the *w* loci in both the $+^c$ and $+^o$ chromosomes was in the ratio of 4:1. This finding has some relevance for our definition and characterization of the gene. From the operational point of view genes can be characterized by their recombinational relationships, by their functional attributes and by their mutational rates. Previously it has been demonstrated that the recombinational separation of the *w* mutants to left and right halves is paralleled precisely by the *z* reaction of the mutants with the *z* suppressors in the right half, the non-suppressors in the left (Green, 1959*a*). These facts were interpreted to mean that the recombinational separation of the *w* mutants coincides with their functional separation. The data reported here show further that the two halves may be distinguished by the mutation operation. The all-important bearing these facts have on the interpretation of pseudoallelism is almost self-evident. Thus the pseudoallelic components of the *w* loci consist of two groups which can be distinguished by all those operational criteria which can be invoked to distinguish one gene from a non-allelic one. On this basis the conclusion follows that the two halves of the *w* loci are distinctive genes, non-allelic by all current operational criteria. The fact that two independent mutants may manifest the phenotypic criterion of allelism is but a first step in assigning allelic relationships. What the numerous instances of pseudoallelism have so clearly demonstrated is that phenotype as a criterion for allelism is wholly presumptive and cannot stand alone.

5. SUMMARY

1. The X-ray mutability of wild-type isoalleles of the *white-eye* loci in *Drosophila melanogaster* has been determined.

2. No difference in the mutability of the isoalleles was found. However, an appreciable difference in mutation rate of the two halves of the *w* loci was found.

3. The bearing of these data on the problems of isoallelism and pseudoallelism are discussed.

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The cytology of *Aspergillus nidulans*

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INTRODUCTION

In recent years, the cytology of a number of ascomycetes has been studied, using modern staining methods and squash preparations: e.g. *Neurospora crassa* (McClintock, 1945; Singleton, 1953), *Glomerella cingulata* (Wheeler, Olive, Ernest & Edgerton, 1948), *Patella melaloma* (Olive, 1950), *Pyronema confluens* (Wilson, 1952), *Venturia inaequalis* (Day, Boone & Keitt, 1956), *Hypomyces solani* f. *curcubitae* (El-Ani, 1956), *Sordaria fimicola* (Carr & Olive, 1958) and *Nectria peziza* (El-Ani, 1959). This list, though far from complete, reflects the attention that has been paid to species with linear or clavate asci, and particularly to the pyrenomycetes, to the apparent exclusion of the plectomycetes. *Aspergillus nidulans*, the subject of the present investigation, is a plectomycete with a non-linear ascus.

Both haploid and diploid strains of *Aspergillus nidulans* have been investigated. Haploid strains are those in which, as usual in fungi, the nuclei of the vegetative hyphae are all haploid. Diploid strains are heterozygotes made by Roper's (1952) technique; the nuclei of their hyphae are mainly diploid (Pontecorvo & Roper, in Pontecorvo, 1953). The main object of this work was to study the behaviour of the nuclei in asci of diploid strains, but it was necessary to have as a basis for interpretation that in haploid strains. In the primordium of the ascus in haploids, there are two nuclei which fuse to give the zygote nucleus, the only diploid nucleus of the life cycle; it immediately undergoes meiosis. It was desired to find out whether in diploid strains fusion of two diploid nuclei occurs in the ascus primordium, followed by a tetraploid meiosis.

The account of the cytology of the ascus in haploid strains is based mainly on *wild type*. Development of asci has been studied in detail in two diploids. Diploid A, one of the first to be synthesized by Pontecorvo and Roper, has the genotype $\frac{+\ +}{ad2\ y}\ \frac{w1}{+}\ \frac{lys1}{+}$. The genotype of Diploid B is $\frac{ad14\ +\ \ +\ \ +\ bi1}{+\ pro1\ paba1\ y\ +}\ \frac{w3}{+}$. (For symbols etc., see Käfer, 1958.)

CYTOLOGICAL TECHNIQUES

Material was grown at 37° C. in slants of minimal medium (or for auxotrophs, of supplemented minimal) in 6 × 1 inch tubes. The slants were inoculated either with conidia, or in the case of heterokaryons with a block of agar cut from the growing

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front of the heterokaryotic mycelium. First metaphase is observed in wild type 4 days after inoculation of conidia, and in diploids after 5 days.

A block of agar with perithecia (or more strictly, cleistocarps) of the age required for cytological examination was cut out of the slant and fixed in freshly mixed 1 : 3 acetic alcohol at room temperature. The material was left in the fixative and stored in a refrigerator. The best preparations were made from freshly fixed material, but satisfactory preparations have been made from material stored for 6 weeks.

The only staining procedure found to be consistently satisfactory was as follows. A piece of agar with the perithecia on it was removed from the fixative and hydrolysed for 5 minutes in normal hydrochloric acid at 60° C. Perithecia were then scraped off the agar block and placed in a drop of aceto-orcein on a slide. A 1% solution of Gurr's synthetic orcein in 50% acetic acid was used. The contents of the perithecia were pressed out with a needle, and the wall debris removed. The material was spread by tapping the cover slip, and the preparation squashed and sealed.

Most of the photographs were taken using a Cooke Troughton and Simms 80 × apochromatic objective and a Leica camera with 'Mikas' attachment. The film was Ilford 'Microneg pan', and an Ilford 'micro-green' filter was used. Some were taken with a Bausch and Lomb 90 × apochromat and a Leitz 'Makam' on Kodak contrast process panchromatic sheet film. All the drawings have been made from photographs. The magnification in all illustrations is × 2500, unless otherwise stated.

HAPLOID STRAINS

Asci in haploid strains arise from croziers. The two nuclei of the terminal cell of an ascogenous hypha divide simultaneously (Figs. 28–29) and a uninucleate tip cell, a binucleate penultimate cell and a uninucleate basal cell are formed (Figs. 4, 30). The penultimate cell enlarges to form the ascus; its two nuclei fuse to give the diploid zygote, which immediately undergoes meiosis (Figs. 4, 30, 5, 25, 31). The tip and basal cells fuse to give a binucleate cell, which forms a second crozier (Fig. 1). The process may be further repeated.

In prophase of the first meiotic division in haploid strains, the chromosomes form too dense a mass for the details of their structure and pairing behaviour to be made out. In early prophase fine threads are seen extending free from the mass. In pachytene a large knob is often conspicuous (Figs. 7, 31). By restaining the preparation with propiono-carmine, so that the nucleolus, which is unstained in the orcein preparations, could be seen, it was shown that this knob is the satellite of the nucleolus-organiser chromosome. Many of the chromosomes have prominent terminal chromomeres situated against the nuclear membrane.

While it appears that in pachytene the chromosomes are fully paired throughout their length, by early diakinesis the two chromosomes of the bivalent are often widely separated; generally they are in contact only at one end, forming a V (Figs. 8, 2). During diakinesis they again pair, so that in late diakinesis the

bivalent appears as two rods lying parallel to each other without any evidence of chiasmata (Fig. 2).

At diakinesis eight bivalents are seen. They differ somewhat in length, and are

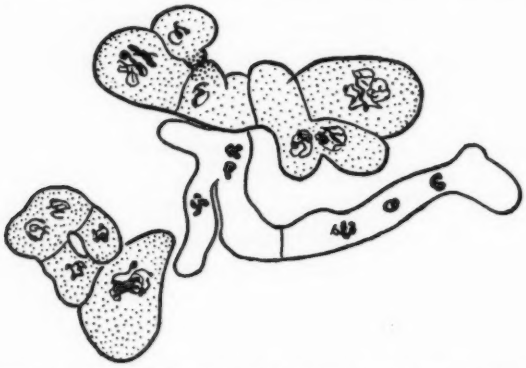


Fig. 1. *Wild type.* ×2000. Three asci with stages in the proliferation of croziers. At *top left*, the tip and basal cells of the crozier are beginning to fuse. *Top right*, binucleate cell resulting from fusion of tip and basal cell. *Bottom left*, secondary crozier with uninucleate tip cell, binucleate penultimate cell and uninucleate basal cell, still attached to ascus formed by primary crozier.

either bent or show a constriction at a characteristic position, which may indicate the position of the centromere (see Figs. 3, 35*a*).

Chromosome 1, the longest chromosome, is frequently curved or bent near the middle in mid-diakinesis.

Chromosome 2 is about the same size as Chromosome 1, and has a satellite about

Fig. 2. Diagram showing appearance of a bivalent in early diakinesis, late diakinesis and first metaphase.

one-fifth of the length of the whole chromosome at late diakinesis. It is frequently bent in mid-diakinesis at the satellite constriction.

Chromosome 3 is slightly smaller than Chromosomes 1 and 2, and is bent at mid-diakinesis in a sub-median position.

Chromosomes 4 and 5 are rather smaller than Chromosomes 1 to 3. Both have sub-terminal constrictions.

Chromosomes 6 and 7 are smaller than Chromosomes 4 and 5. One at least has a sub-terminal constriction.

Chromosome 8, the smallest chromosome, is rather smaller than Chromosomes 6 and 7.

In general, the chromosomes may be classified at first metaphase as three large,

two medium sized and three small. Often, however, the size differences are not very apparent at first metaphase. They are generally much more distinct at late diakinesis (Figs. 34, 35, 35*a*, 3). The eight bivalents at first metaphase are shown in Figs. 10–13, 36–37.

The first metaphase spindle is narrow with flattened ends (Fig. 42). The bivalents are attached to the outside of the spindle, forming a ring in polar view (Fig. 36). Their centric regions are drawn out, converting the parallel rods of diakinesis into a round or diamond shaped figure in side view (Figs. 12, 2).

First anaphase is shown in Figs. 38–39. The three smallest chromosomes may disjoin early; the difference in size between Chromosome 8 and Chromosomes 6 and

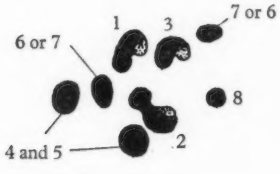


Fig. 3

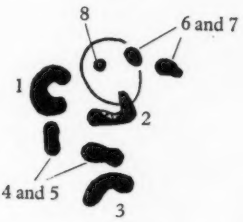


Fig. 35*a*

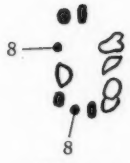


Fig. 15*a*

Fig. 3. *Wild type.* × 6000. Mid-diakinesis, showing differences in size between the eight chromosomes.

Fig. 35*a*. *pro*1 *paba*1 *y* × *ad*14 *bi*1; *w*3. × 5000. Mid-diakinesis. Drawing of cell shown in Fig. 35. In the photograph Chromosome 4 (or 5) appears to be touching Chromosome 1.

Fig. 15*a*. *Wild type.* Drawing of cell shown in Fig. 15. Univalents (early anaphase) in black, bivalents in outline. The bottom right univalent is partly obscured by one of the bivalents in the photograph.

7 is then apparent (Figs. 15, 15*a*). In the second meiotic division, and in the mitotic division which follows meiosis (third ascus division), the chromosomes are very small, and nothing can be seen of their morphology; indeed, they can hardly ever be counted even subsequent to first anaphase. The interphase nuclei are however quite large (Figs. 18, 22).

At late anaphase of all three divisions, two rings are seen in which the individual chromosomes cannot be distinguished (Figs. 16, 17, 21), and a thread, presumably representing the spindle, often connects them (Figs. 16, 24). The division of the nuclei of an ascus is synchronized (Figs. 17, 19–21, 23–24).

Centrioles cannot be demonstrated by the staining methods used. During ascospore formation the cytoplasm appears to be divided by cleavage about each

EXPLANATION OF PLATES

Plate I, Figs. 4–16. *Wild type.*

Fig. 4. Ascus with nucleus in early meiotic prophase; and secondary crozier with uninucleate, binucleate and uninucleate cells.

Fig. 5. Three asci showing progressive stages from fusion nucleus to pachytene, the ascus enlarging meanwhile.

Fig. 6. Pachytene.

Fig. 7. Pachytene, with conspicuous knob at 10 o'clock (*arrow*).

Fig. 8. Early diakinesis. Note V-shaped appearance of two bivalents at right of nucleus (*arrows*).

Fig. 9. Mid-diakinesis.

Figs. 10–13. First metaphase; early stages with eight bivalents spread out along the length of spindle.

Fig. 14. First metaphase; late stage with bivalents arranged at equator of spindle.

Fig. 15. Early first anaphase, with the three smallest pairs of chromosomes already disjoined. See drawing, Fig. 15*a*.

Fig. 16. Late first anaphase, showing chromosomes clumped into two rings joined by a thread (spindle).

Plate II, Figs. 17–27. *Wild type.*

Fig. 17. Late first anaphase (*left* ascus) and late second anaphase (*right*).

Fig. 18. First interphase.

Fig. 19. Second prophase (*left*); early first prophase (*right*).

Fig. 20. Second metaphase.

Fig. 21. Late second anaphase.

Fig. 22. Second interphase.

Figs. 23–24. Third anaphase.

Fig. 25. *Top left:* third prophase. *Centre:* early stage in formation of ascospores. *Bottom:* third telophase. *Top right:* ascus with nucleus in early first prophase and to left of it crozier with fusion nucleus in penultimate cell.

Fig. 26. Ascus (ruptured) with uninucleate spores with well developed walls.

Fig. 27. Mature binucleate spores.

Plate III, Figs. 28–43. (Figs. 28–41. *pro*1 *paba*1 $y \times ad14$ *bi*1; *w*3)

Fig. 28. Prophase of division of two nuclei of young crozier.

Fig. 29. Late anaphase of division of nuclei in young crozier.

Fig. 30. *Right:* ascus with nucleus in early first prophase. *Left:* secondary crozier with uninucleate, binucleate and uninucleate cells.

Fig. 31. *Right:* pachytene, showing knob at 3 o'clock (*arrow*). *Left:* crozier with uninucleate tip cell, penultimate cell with fusion nucleus, and uninucleate basal cell.

Fig. 32. Diplotene

Fig. 33. Mid-diakinesis.

Figs. 34–35. Diakinesis, showing differences in size between chromosomes. See drawing, Fig. 35*a*.

Fig. 36. First metaphase in polar view.

Fig. 37. First metaphase in side view.

Fig. 38. Early first anaphase; chromosomes disjoining simultaneously.

Fig. 39. Mid-first anaphase. Eight chromosomes could be counted in upper figure.

Fig. 40. Third metaphase.

Fig. 41. Delimitation of ascospores, each potential spore with single nucleus.

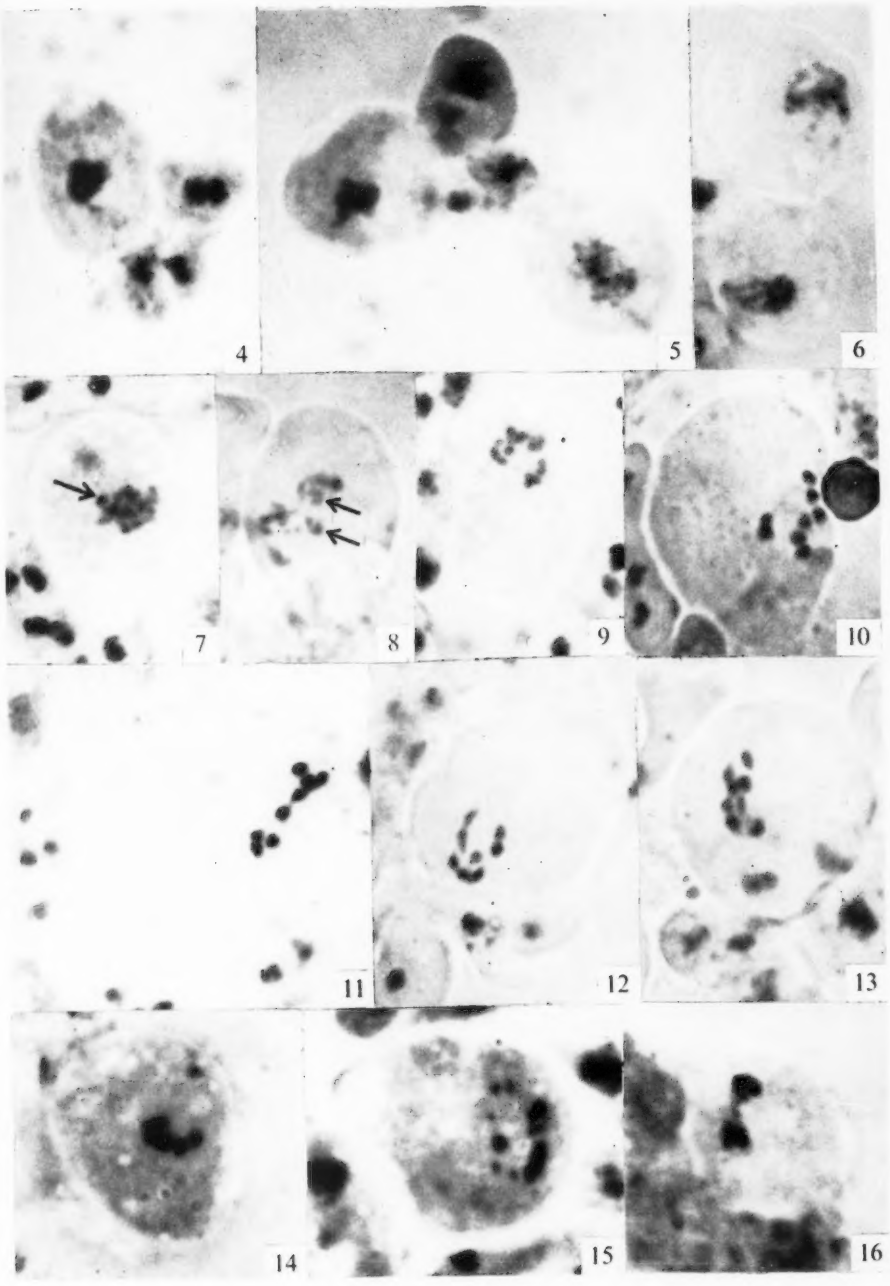
Fig. 42. Diploid A. First metaphase, showing spindle.

Fig. 43. *Wild type.* Ascus with a 'blob,' presumably the result of clumping of chromosomes at first metaphase.

Plate IV, Figs. 44 and 45.

Fig. 44. *Wild type.* Squashed perithecium. × 800.

Fig. 45. Diploid A. Squashed perithecium. × 800.



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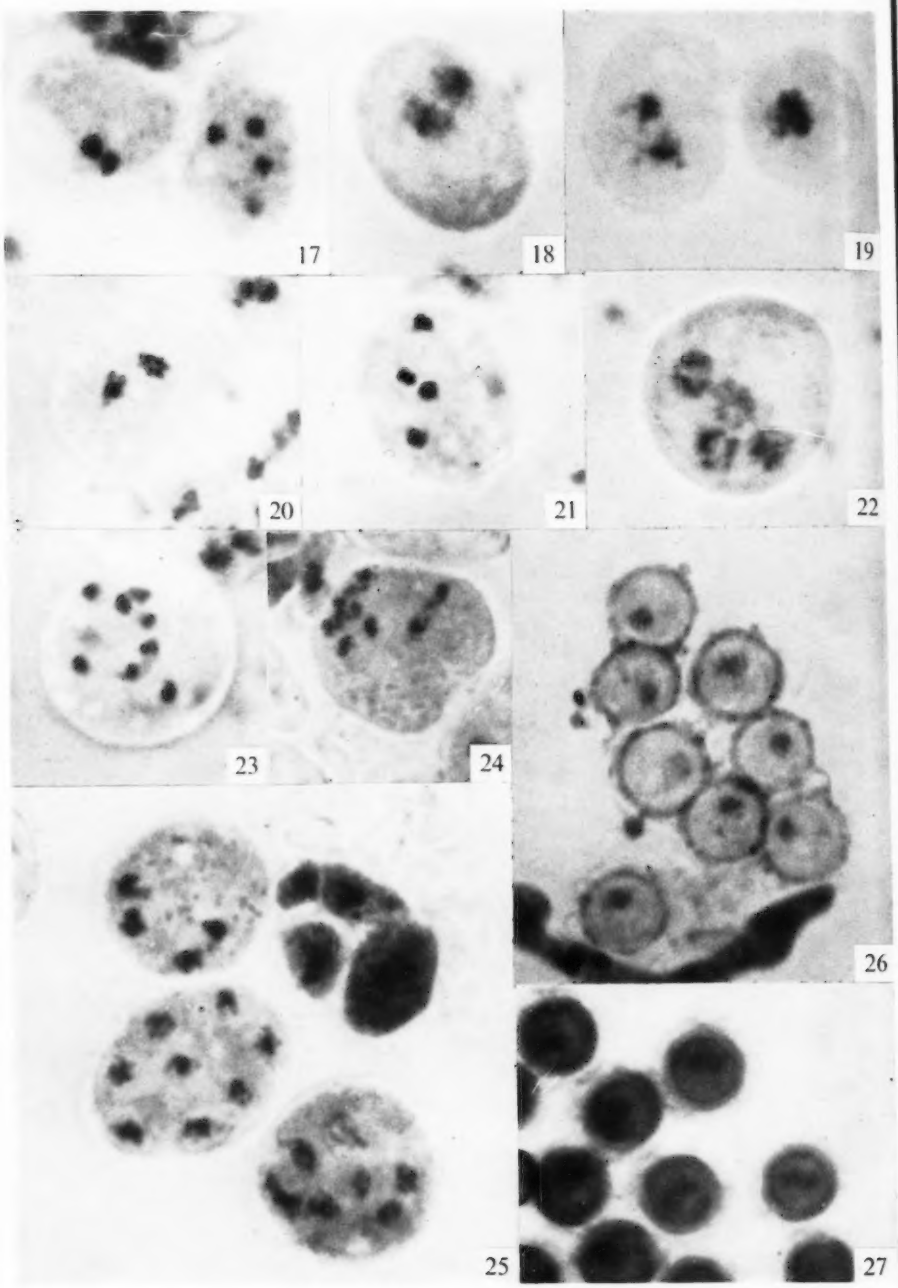
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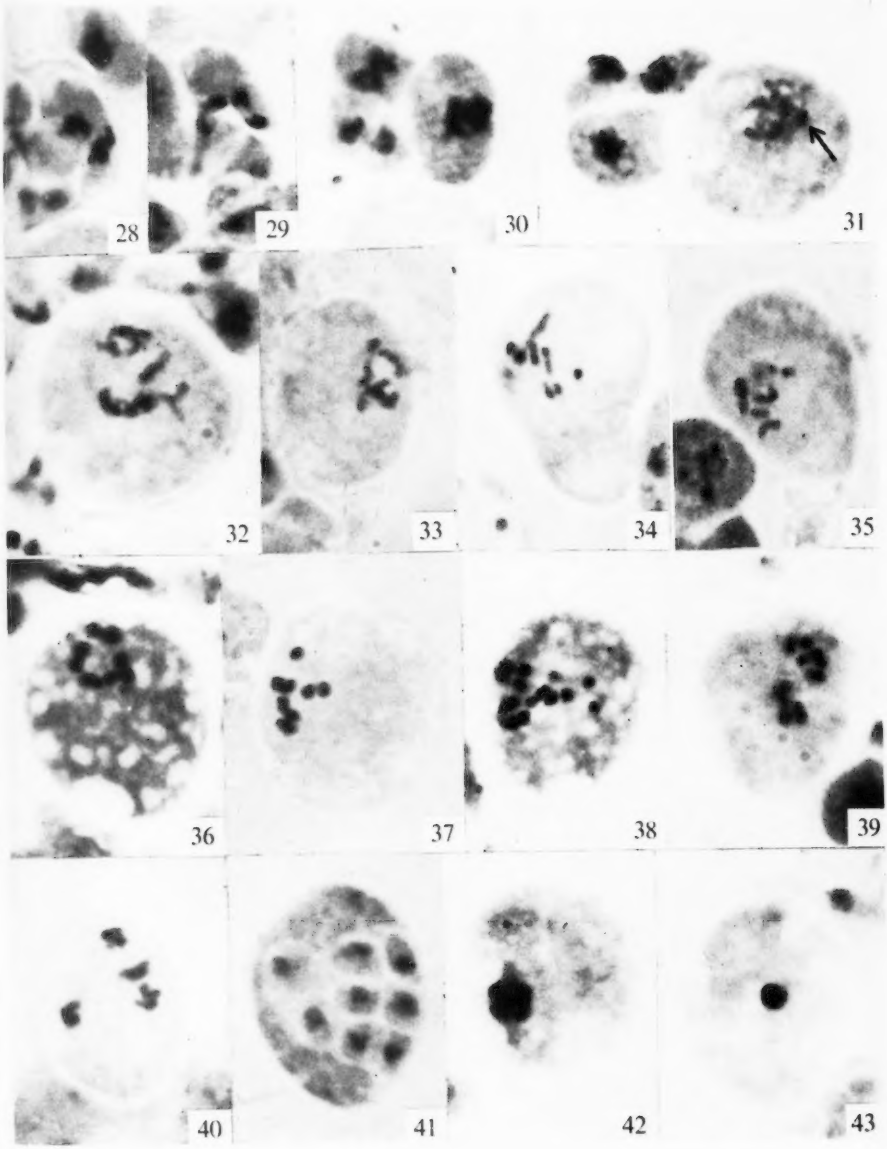
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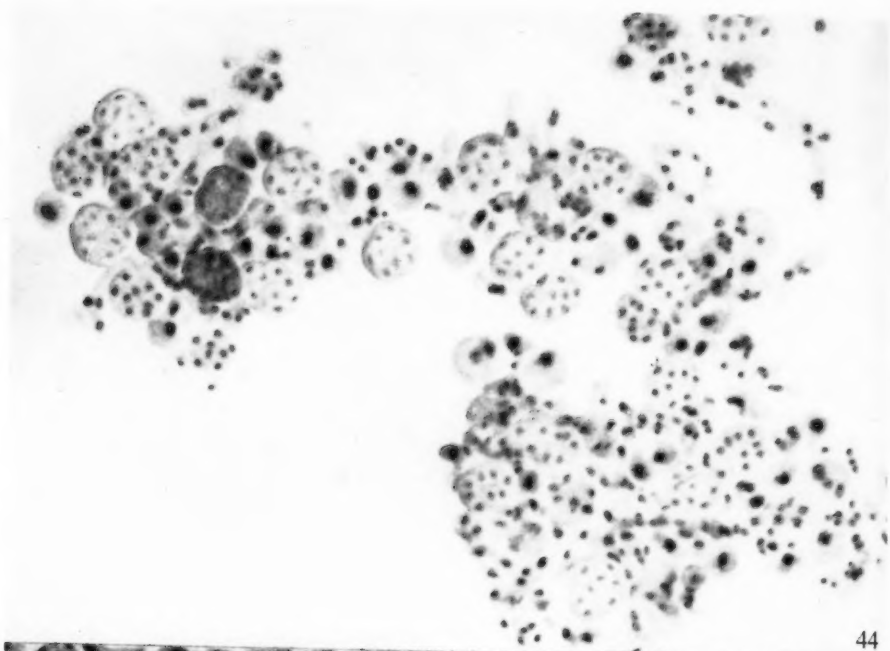

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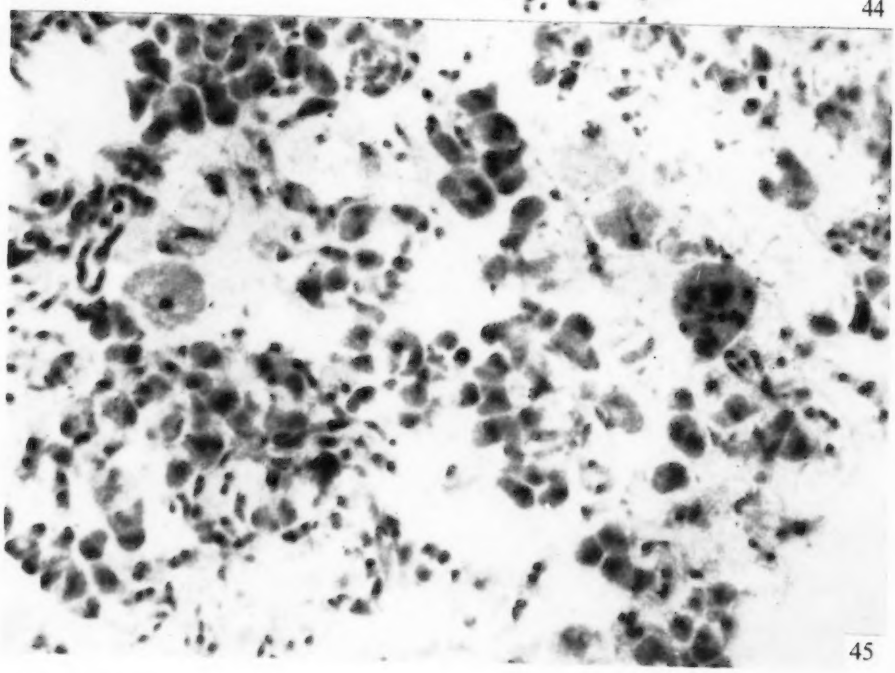

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of the eight nuclei, forming eight uninucleate spores. Generally not all the cytoplasm is included in the spores (Fig. 41). As the sculpturing of the wall of the ascospore develops, the contents are obscured, but later a single nucleus may again be seen inside (Fig. 26). Some time afterwards a further mitosis takes place, so that the spores are binucleate when mature (Adam, in Pontecorvo, 1953) (Fig. 27).

The above account refers principally to *wild type* (Figs. 4–27). The heterokaryon between *pro*1 *paba*1 *y* and *ad*14 *bi*1 ; *w*3, the two haploid strains from which Diploid B was synthesized (Figs. 28–41), differs in no essential cytological details from wild type, but there may be a greater number of sterile hyphae.

DIPLOID STRAINS

The general appearance of a preparation of the contents of a perithecium(cleistocarp) of a haploid strain, at the stage when the perithecial wall is beginning to turn dark in colour, is shown in Fig. 44. There are many asci in all stages of development from early meiotic prophase to the commencement of ascospore formation,

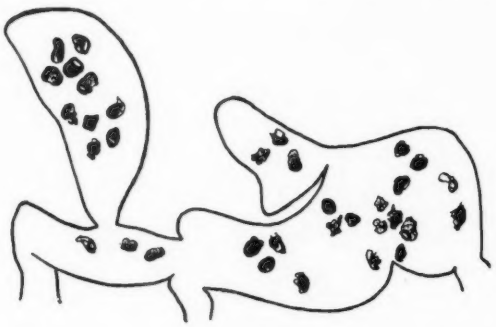
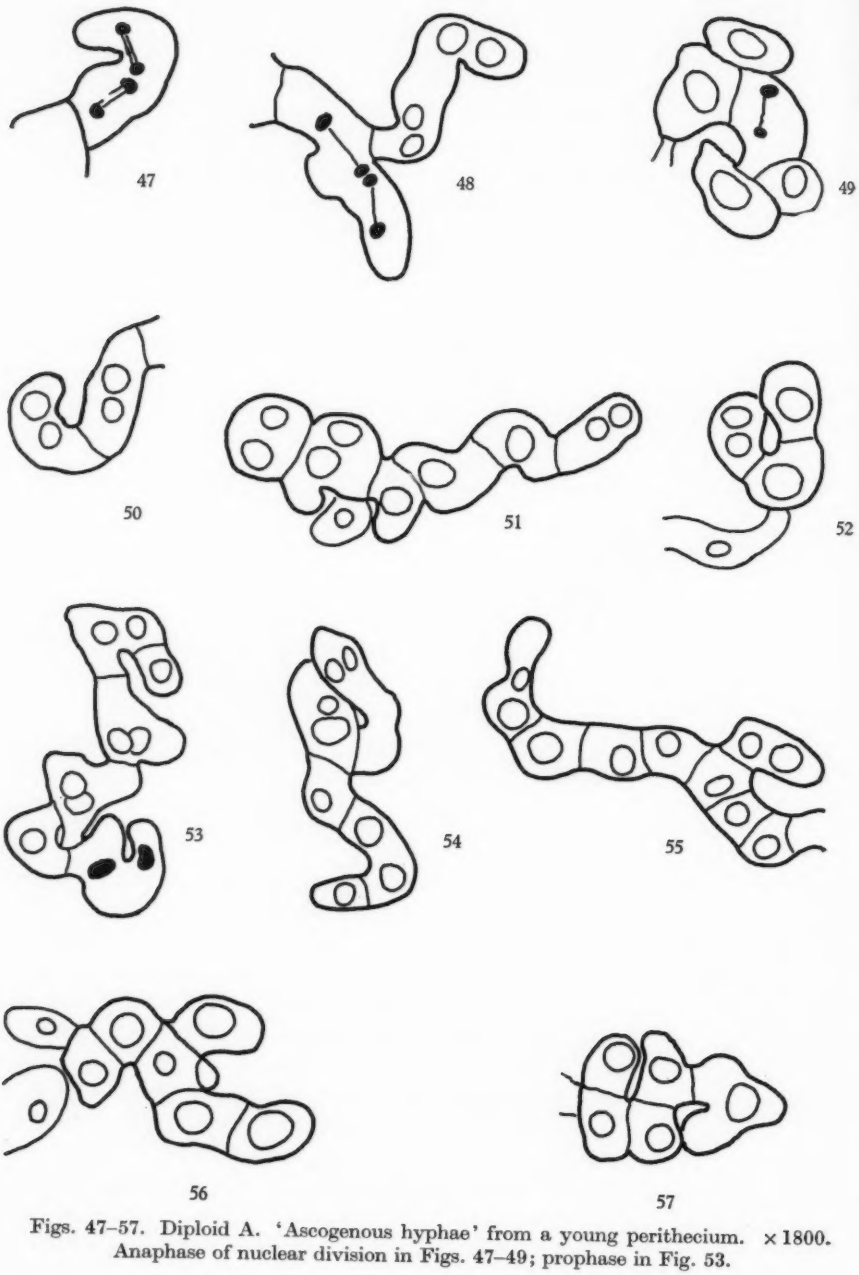


Fig. 46. Diploid A. Inflated sac of sterile hypha. × 1500.

and very few sterile hyphae. A preparation of the perithecial contents of a diploid strain at the same stage, as judged by the colour of the perithecium wall, is strikingly different, as shown in Fig. 45. There are many more sterile hyphae. These have little cytoplasm, small dense nuclei, and are irregular in shape; they may be inflated into large multinucleate sacs (Fig. 46). There are relatively few asci, and those present contain nuclei in pachytene or diakinesis and first metaphase, or have a single darkly staining blob. Typical stages later than first metaphase are very infrequent, and asci with spores are rarely seen. There are also coiled hyphae with densely staining cytoplasm and large diffuse nuclei with a large nucleolus (Figs. 58–59)—quite unlike anything in haploid strains.

In young perithecia, before any asci are recognizable, these coiled hyphae with dense cytoplasm may be distinguished from the sterile hyphae, and at this time they have both binucleate and uninucleate cells. In older perithecia, their cells are mostly uninucleate. The nuclei of both binucleate and uninucleate cells may divide (Figs. 47–49, 53). The terminal cell and penultimate cell of a hypha may



Figs. 47–57. Diploid A. 'Ascogenous hyphae' from a young perithecium. ×1800. Anaphase of nuclear division in Figs. 47–49; prophase in Fig. 53.

both be binucleate (Fig. 50); these are evidently derived from the quadrinucleate state following nuclear division in a binucleate cell (Fig. 48). The terminal cell may be binucleate and the penultimate uninucleate (Figs. 51, 55), or the terminal cell may be uninucleate and the penultimate binucleate (Figs. 53, 54). Even when a hypha is curved at the tip and has uninucleate, binucleate and uninucleate cells in sequence, it does not look like the crozier of the haploid strains. The asci of diploid strains, it appears, do not develop from croziers, but from some of the uninucleate cells of the coiled hyphae.

That the ascus develops from a cell with a single (diploid) nucleus is indicated by observations on later stages. *The chromosome configurations seen at diakinesis and first metaphase in diploid strains are the same as those in haploids.* In Diploid B, eight normal bivalents are seen (Figs. 64–66). Diploid A has a fragment (see below), and the usual configurations are eight bivalents and a fragment either free or associated with one of the bivalents (Figs. 68–71)—the same as seen in the cross between the haploid strains from which the diploid was synthesized.

The bivalent structure of the chromosomes in diploid strains is clearly evident at diakinesis and first metaphase; indeed their appearance at diakinesis as two parallel rods is often more conspicuous in diploids than in haploids. At pachytene also the chromosomes are often much more clearly seen than in haploid strains at that stage. This may be due to the size of the asci in diploid strains; they are larger and more rounded in outline than those of haploids (Table 1; compare Figs. 8–15 and 33–39 with Figs. 64–66 and 68–72, which are all at the same magnification). The chromosomes themselves are not, however, noticeably larger in diploid than in haploid strains.

Table 1. *Dimensions of squashed asci at diakinesis–first metaphase*

	No. of cells	Mean diameter (μ)			Ratio length/breadth		
		Range	Mean	Standard deviation	Range	Mean	Standard deviation
Haploids	60	9–15	11·6	1·31	1·00–1·85	1·28	0·175
Diploids	65	10–16	13·9	1·34	1·00–1·56	1·14	0·108

The stages most frequently seen are pachytene and diakinesis–first metaphase. Asci with two or four nuclei, or showing the second and third nuclear divisions, or containing ascospores are rare; much less frequent, even in older perithecia, than would be expected from the number of first metaphases. This suggests that many asci degenerate at first metaphase. It is presumed that Fig. 61 (a normal first metaphase in polar view), and Figs. 62 and 63 represent three stages of degeneration. Meiosis proceeds normally up to first metaphase (though possibly more slowly than in haploid strains), but first anaphase frequently fails to occur, and the chromosomes clump together to form a darkly staining 'blob' (Fig. 63). These blobs are a conspicuous feature of older perithecia. They are not seen in young perithecia with only a few asci, and they are seen only in large asci, as large as or larger than asci at diakinesis and first metaphase.

These dense blobs are occasionally seen in haploids (Fig. 43). One is illustrated by Pontecorvo (1953, Fig. 4*b*), who considered it to be the zygote nucleus. In his figure, the ascus is too large for this to be the correct interpretation. Blobs may be more frequent in some haploid auxotrophic strains than in wild type, but even so are much rarer than in diploids where they are extremely common.

Soon after any spores formed are mature, the contents of other asci and of the coiled hyphae degenerate, and one then finds some straight hyphae, very like those of vegetative mycelium, in the perithecium, together with a few ascospores and a lot of detritus.

In the first diploids synthesized, asci with 16 spores were frequently seen; 8-spored asci were found with them in the same perithecium (Pontecorvo & Roper, in Pontecorvo, 1953). I have found 16-spored asci to be somewhat rarer than is suggested by Pontecorvo and Roper. The majority of asci in Diploid A were 8-spored; and in Diploid B I have seen no 16-spored asci at all. Nothing definite has been discovered about their origin.

EXPLANATION OF PLATES

Plate V, Figs. 58–66.

Figs. 58–59. Diploid A. Overstained preparation showing coiled hyphae with dense cytoplasm and sterile hyphae with little cytoplasm. × 1800.

Fig. 60. Diploid A. Cells of coiled hyphae enlarging and becoming asci.

Fig. 61. Diploid B. First metaphase in polar view.

Fig. 62. Diploid A. Chromosomes at first metaphase clumping into a ring.

Fig. 63. Diploid B. 'Blob' of clumped chromosomes.

Figs. 64–66. Diploid B. First metaphase showing eight bivalents.

Plate VI, Figs. 67–80. (Figs. 67–72. Diploid A).

Fig. 67. Diakinesis.

Fig. 68. First metaphase, showing single free fragment towards one pole of the spindle.

Figs. 69–70.* Late diakinesis, showing single fragment associated with Chromosome 6 and seven other bivalents.

Fig. 71.* Single fragment associated with Chromosome 6 (*centre bottom*), six other bivalents and (*top*) pair of Chromosome 8 univalents.

Fig. 72.* Two fragments, one associated with Chromosome 6 and one free (at 4 o'clock); seven other bivalents.

Figs. 73–74.* *ad*2 *y*. Diakinesis with nine bivalents.

Figs. 75–80. *ad* 2 *y* × *ribo*1 *bi*1.

Fig. 75.* First metaphase, showing single fragment associated with Chromosome 6.

Fig. 76. Ascus with four normal ascospores at early stage of wall formation and four nuclei (in two pairs) not in normal spores.

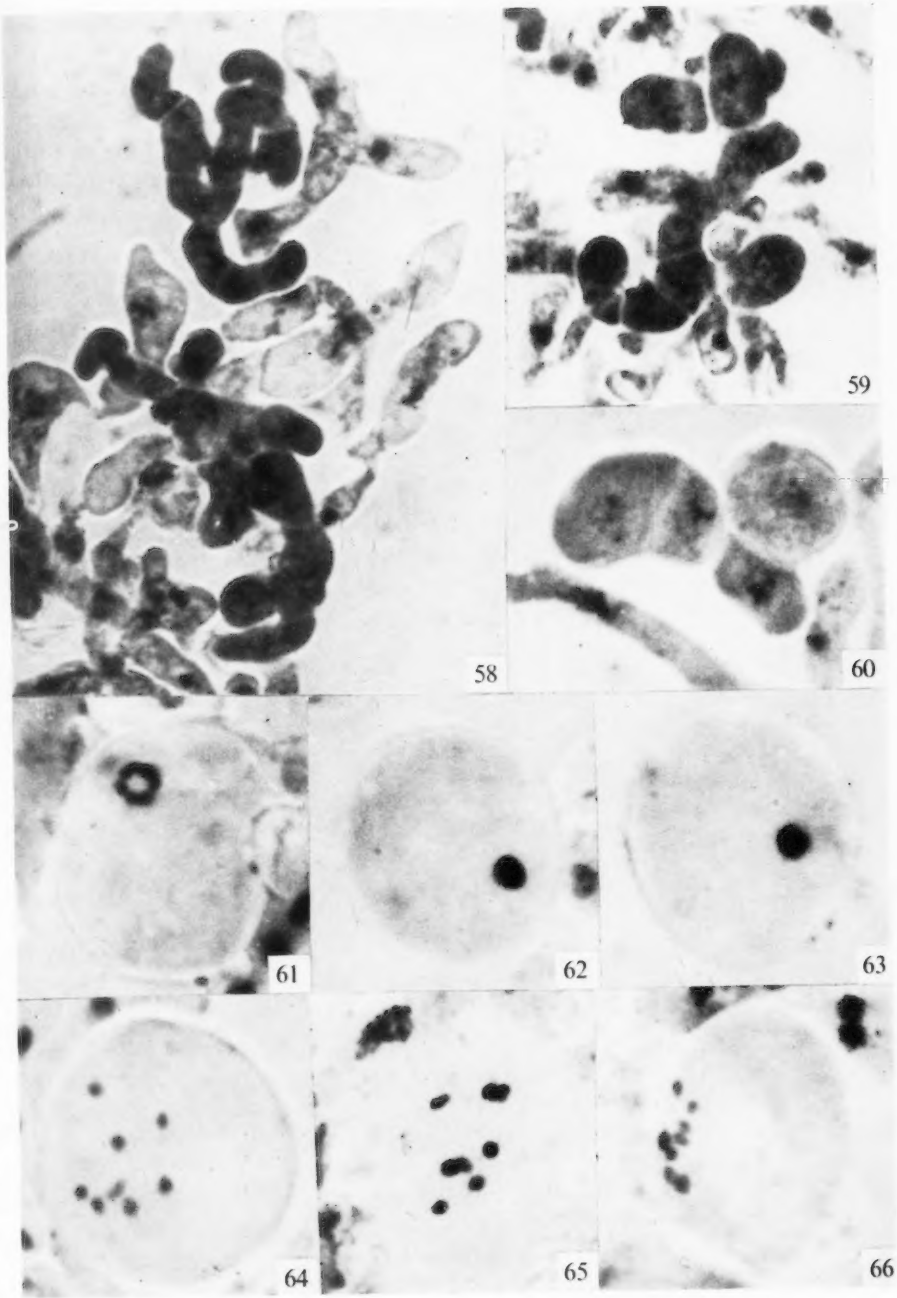
Fig. 77. Mature ascus with four normal spores and two small degenerate spores.

Fig. 78. Mature ascus with three normal spores and four small degenerate spores.

Fig. 79. Mature ascus with one normal spore and five degenerate spores.

Fig. 80. Ascus with two normal anaphase figures and a scattered group of chromosomes.

* See drawings, Figures 69*a*–72*a*, 73*a*–74*a*, 75*a*.



C. G. ELLIOTT

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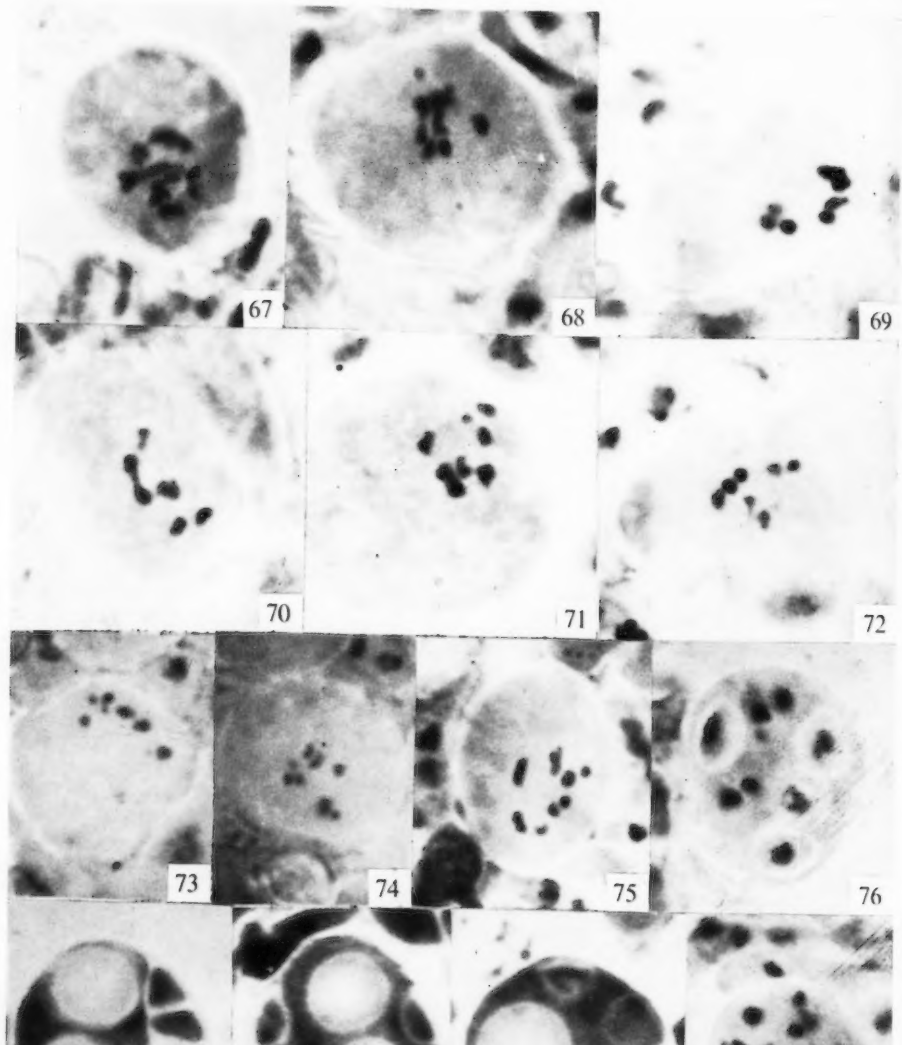
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The observation that in diploid strains the ascus has a single diploid nucleus which undergoes meiosis predicts that ascospores should be haploid. This expectation is born out by the ploidy of cultures derived from ascospores isolated by micromanipulation from perithecia of two diploids. From one diploid, 151 spores were isolated and 54 grew. From the other, that called Diploid B and studied in detail cytologically, 416 spores were isolated and 38 grew. Some cultures were derived from spores dissected from 8-spored asci, but most were from free spores. Of the 92 cultures, 91 were haploid (so classified according to the diameter of their conidia; see Pontecorvo, Tarr Gloor & Forbes, 1954) and one, from Diploid B, was diploid. The latter culture was, like the parent diploid, a green prototroph, and analysis of mitotic segregants from it showed that its genotype was the same as that of the parent strain. It may well have been a conidium isolated by mistake.

THE *DIPLOID A* FRAGMENT

In Diploid A eight bivalents and a fragment were observed at diakinesis and first metaphase. The fragment was either free (Fig. 68) or more often associated with one of the larger of the three small chromosomes (Figs. 69–71, 69*a*–71*a*). The chromosome with which the fragment pairs will be defined as Chromosome 6.

Diploid A was synthesized from the two haploid strains *ad*2 *y* and *w*1 *lys*1. The heterokaryon between these two strains was made and perithecia from it examined. These perithecia had, of course, the morphology of perithecia of a single haploid strain. The chromosome configurations seen at first metaphase were the same as in Diploid A, a single fragment being either free or associated with Chromosome 6.

In a homothallic fungus, the perithecia which develop on heterokaryons may arise from one or other of the component strains, or from both (hybrid perithecia). When the strains in a heterokaryon differ by conidial colour markers, the kinds of perithecia may be identified by streaking ascospores from them on complete medium and observing the colours of conidia which develop (Hemmons, Pontecorvo & Bufton, in Pontecorvo, 1953). The heterokaryon *ad*2 *y* + *w*1 *lys*1 was found to give a rather low proportion of hybrid perithecia; some others gave a very high proportion. The hybrid perithecia produced by *ad*2 *y* + *w*1 *lys*1 (and similar heterokaryons) growing in slants were larger and developed earlier than the selfed perithecia, and hence they could be selected for cytological examination. It was shown by Hemmons *et al.* and by Strickland (1956) that in hybrid perithecia the vast majority of *asci*, if not all of them, are hybrid (cf. Olive, 1954). Hence one can be confident that the chromosomes examined are those of hybrid asci.

In Diploid A, two fragments were sometimes present. In such cells, one fragment was as a rule associated with Chromosome 6 and the other was free (Figs. 72, 72*a*). Both in this case, and when there was only one fragment, the other chromosomes had their normal bivalent appearance. Presumably the fragment may undergo mitotic non-disjunction. The similarity in appearance of first metaphases in Diploid A and the heterokaryon, and of the chromosomes other than Chromosome 6 in cells with one and with two fragments is good evidence that in the asci of diploid strains a diploid, not a tetraploid, nucleus undergoes meiosis.

The strains from which Diploid A was made were examined separately, and the heterokaryons between each and a third strain, *ribo*1 *bi*1, were studied, to find out which strain contributed the fragment. In *ad*2 *y*, which is self-fertile, nine bivalents are normally seen at first metaphase (Figs. 73–74, 73*a*–74*a*). One of them is about half the size of the smallest of the other eight. (In Fig. 71 (from Diploid

Fig. 69*a* Fig. 70*a* Fig. 71*a* Fig. 72*a*

Figs. 69*a*–72*a*. Diploid A. Drawings of cells shown in Figs. 69–72. *f* indicates fragment.

A) the fragment is seen to be about half the size of the Chromosome 8 univalents.) In about one-fifth of cells, eight bivalents and two small univalents (presumably) were present. No abnormal asci were observed in *ad*2 *y*, apart from one with eleven spores. In the heterokaryon *ad*2 *y*+*ribo*1 *bi*1 the configurations observed at first metaphase (cf. Fig. 75) were the same as in the heterokaryon *ad*2 *y*+*w*1 *lys*1. Highly abnormal asci were found in the perithecia from these two heterokaryons.

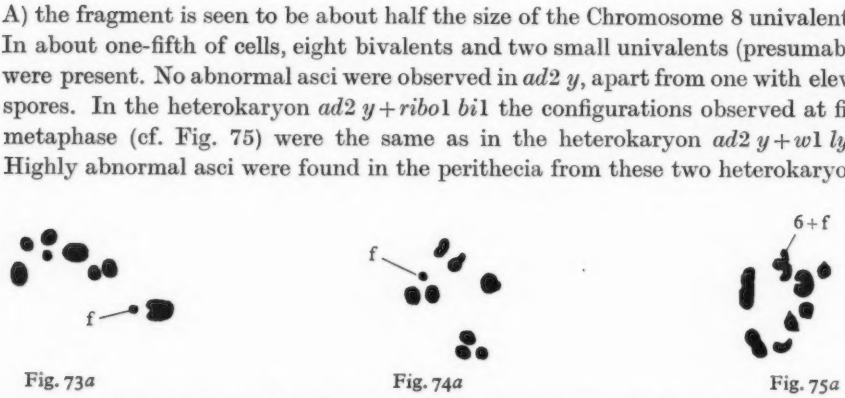

Fig. 73*a* Fig. 74*a* Fig. 75*a*

Figs. 73*a*–74*a*. *ad*2 *y*. Drawings of cells shown in Figs. 73–74. Smallest (fragment) bivalent indicated by *f*.

Fig. 75*a*. *ad*2 *y* × *ribo*1 *bi*1. Drawing of cell shown in Fig. 75. Single fragment associated with Chromosome 6 (6+*f*).

Many spores were abortive; and the number was frequently less than eight (Figs. 76–79 and Table 2). Asci with less than eight spores are the result presumably of some nuclei of an ascus failing to divide properly: Fig. 80 shows an ascus with two anaphase figures and an irregularly scattered group of chromosomes. Similar abnormal asci occur in Diploid A.

The strain *w*1 *lys*1 is completely self-sterile, and no perithecia were formed by the heterokaryon between it and *ribo*1 *bi*1. However, since the heterokaryons *ad*2 *y*+*w*1 *lys*1 and *ad*2 *y*+*ribo*1 *bi*1 were similar, it is clear that the fragment in Diploid A is contributed by *ad*2 *y*. No abnormal asci were found in crosses between *ribo*1 *bi*1 and *pro*1 *paba*1 *y* (a 'normal' strain—one of the parents of Diploid B).

Table 2. *Abnormal asci in the cross* ad2 y × ribo1 bi1

Number of										
normal spores	8	7	6	6	5	5	5	4	4	
abnormal spores	–	–	–	2	3	2	1	4	2	
Asci observed	29	3	5	17	2	3	3	2	19	
normal spores	3	3	3	3	3	2	2	2	1	1
abnormal spores	5	4	3	2	1	5	4	2	4	3
Asci observed	2	2	2	1	1	3	1	2	1	2

The strain *ad2 y* was obtained by irradiation of *y* with X-rays. *y* has the normal complement of eight chromosomes. The persistence of the fragment in *ad2 y* through many mitotic divisions indicates that it is necessary for viability, or at least that its presence confers some advantage; but the precise nature of the change which occurred in Chromosome 6 cannot be stated on the evidence available. One possibility is that concurrently with the mutation to adenine dependence, Chromosome 6 was broken at the centromere and in one arm, giving rise to a centric fragment and a chromosome with a deletion. If this were so, the configuration seen in Diploid A would be an association of the normal Chromosome 6, the deficient chromosome and the fragment. Heterokaryon tests (Pritchard, 1955) indicate that *ad2* is allelic with *ad9*, which is located in Linkage Group I. Pritchard (1955) found abnormally low recombination fractions in crosses between *ad2 y* and 'normal' strains marked only in Linkage Group I. The information available however is not enough to show that Linkage Group I is on Chromosome 6.

DISCUSSION

The present study has shown that the haploid number of chromosomes in *Aspergillus nidulans* is eight. Earlier statements that it is four (Pontecorvo, 1953; Elliott, 1956) are incorrect. It is to be noted that eight linkage groups have been established by mitotic analysis (Käfer, 1958). The chromosomes differ somewhat in length. Analysis of the chromosomes at pachytene should be possible in diploids, though this is scarcely feasible in haploids.

Normally the diplophase in the life history of *Aspergillus* is confined to the single fusion nucleus in the young ascus. But in addition to this normal cycle, there is a 'parasexual cycle' (Pontecorvo, 1954) with a prolonged diplophase. The diplophase begins with fusion of haploid nuclei in the vegetative mycelium; it ends with mitotic breakdown of diploid nuclei through aneuploidy (Käfer, 1957) to haploidy, and, as now shown, by meiosis.

It has been shown that in the development of asci in diploid strains, the young ascus has a single diploid nucleus which undergoes meiosis, giving haploid ascospores. The most convincing evidence for this apogamous development of asci lies in the chromosome configurations themselves: they are the same as seen in the cross between the two haploid strains from which the diploid was synthesized. The structure of the chromosomes as bivalents is clearly recognizable at late dia-

kinesis in the diploids. No evidence of a tetraploid meiosis has been found, although this cannot be excluded as a rare event.

The diploids differ essentially from haploids in the absence of true croziers and presence of the coiled hyphae with dense cytoplasm. These hyphae, which may be called 'ascogenous hyphae', are made up mainly of uninucleate cells. The variability of the positions in which binucleate cells occur in them suggests that wall formation is not precisely controlled. Each of the cells is potentially an ascus: all the nuclei of the 'ascogenous hyphae' indeed resemble meiotic prophase nuclei in appearance. Some of the cells, particularly in lateral or terminal positions, enlarge (Fig. 60) and become asci. Binucleate (as well as uninucleate) cells are sometimes seen rounding off in this way, but the stages expected, were they to develop further, have not been seen.

One of the remarkable things about the diploids is their sterility. Some haploid auxotrophic strains are self-sterile, producing few small perithecia if any, but they may cross readily with other strains, producing, as with self-sterile strains, perithecia packed with asci and having very few sterile hyphae. Self-sterility in these strains seems to result from a failure of perithecium initiation. In contrast, both the diploids studied produce perithecia in great numbers: the sterility arises as a consequence of processes taking place within the perithecia. In the first place, there are many sterile hyphae in the perithecia, and the 'ascogenous hyphae' bear few asci. The number of asci varies considerably between different diploids; Diploid A has more per perithecium than Diploid B, but they are always few compared with haploid strains. Secondly, many asci degenerate at first metaphase. The numbers of asci in pachytene and diakinesis–first metaphase suggest that meiosis proceeds normally up to the latter stage. But compared with the number of first metaphases, very few asci in subsequent stages and with spores are seen. First anaphase frequently fails, and the chromosomes clump together.

The physiological basis of the sterility of the diploids is an intriguing problem. There is a striking difference in fertility between a diploid strain and the cross between its haploid parents. The diploid state of the mycelium itself must somehow be inimical to the proper functioning of the sexual processes.

SUMMARY

In haploid strains of *Aspergillus nidulans*, asci arise from croziers. The two nuclei of the young ascus (the penultimate cell of the crozier) fuse, and the zygote nucleus immediately undergoes meiosis. At diakinesis and first metaphase eight bivalents are seen: three large (one, Chromosome 2, with a satellite), two medium sized (Chromosomes 4 and 5), two small (Chromosomes 6 and 7) and one very small (Chromosome 8). The perithecia of haploid strains are packed with asci and have very few sterile hyphae.

Diploid strains (heterozygotes made by Roper's technique) are very different from haploids in that the perithecia contain many sterile hyphae with little cytoplasm and coiled hyphae with dense cytoplasm made up mainly of uninucleate cells, and there are few asci in a perithecium. Croziers are absent: some of the cells

of the coiled hyphae become asci. At diakinesis and first metaphase, the same chromosome configurations are seen as in a cross between the haploid strains from which the diploid was synthesized; the chromosomes are bivalents. The young ascus thus has a single (diploid) nucleus which undergoes meiosis, and asci develop apogamously. No evidence of nuclear fusion in the young ascus and of a tetraploid meiosis was obtained. Cultures from ascopores isolated by micromanipulation from perithecia of diploids were all haploid. Meiosis appears to proceed normally to first metaphase, but typical later stages, and asci with spores, are rarely seen. First anaphase frequently fails to occur, and the chromosomes clump together.

In the strain *ad2 y*, obtained from a normal strain by X-irradiation, nine bivalents are present, one a very small fragment. In crosses between *ad2 y* and a normal strain, and in a diploid made from them, the fragment pairs with Chromosome 6. Asci in the cross between *ad2 y* and a normal haploid are highly irregular, often with less than the usual eight spores.

I am indebted to Professor G. Pontecorvo, F.R.S., for his interest throughout these investigations. I especially wish to thank Dr Barbara McClintock for her invaluable assistance, advice and criticism, and it is a pleasure to acknowledge the hospitality of the Biological Laboratory, Cold Spring Harbor. This work was supported by a grant from the Nuffield Foundation.

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Sexual dimorphism in mouse gametogenesis

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INTRODUCTION

There is very little information in the literature on the frequency and behaviour of chiasmata in the female mouse. Crew and Koller (1932) give counts of chiasmata at diplotene of forty-two randomly recorded tetrads and at metaphase of five oöcytes. Since their paper was published no other information has appeared on the subject.

The present paper deals with chiasma frequency in female mice. There are several limitations inherent in the material which make this study more difficult and less exact than the corresponding study in males.

The diplotene stage is exceedingly rarely observed in females (Guenin, 1948), and in those few cases in which it has been found, the tetrads could not be satisfactorily analysed. Consequently, after several unsuccessful attempts, the study of chiasma frequency in earlier stages of meiotic division had to be abandoned, and it was necessary to limit the study of chiasmata to the metaphase stage.

MATERIAL AND METHODS

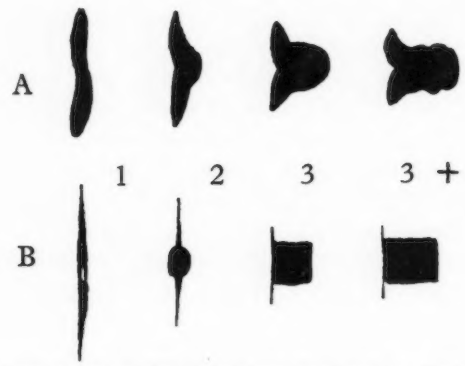
The material for this study was obtained through the kindness of Dr D. S. Falconer, Mr J. Isaacson and Dr M. Latyszewski from the mouse colony in this Laboratory. The material is by no means uniform in any respect; it consists of animals of various ages, strains and outcrosses.

Mice were killed mechanically, and the ovaries were removed as quickly as possible. After the removal of the covering membrane, they were subjected to water pretreatment for 5 minutes. Fixation in acetocarmine was followed by squashing on an albuminized slide. After detachment of the cover slip, the preparation was hydrolysed, stained in a water solution of basic Fuchsin, bleached in sulphurous acid, dehydrated in an increasing series of alcohol solutions and finally mounted in Euparal.

About 10% of metaphases were analysable. It has been found that in searching for metaphases the size of the oöcyte is not a valid criterion, since there is not much correlation between the size of the oöcyte and the stage of meiosis its nucleus can show. To illustrate this point a microphotograph has been taken of two oöcytes of similar size but with different stages of meiosis in them ; one shows the metaphase plate, the other what is probably the end of dictyotene (Plate I, fig. 1).

* Member of external scientific staff, Medical Research Council.

The diagram (Text-fig. 1 and Plate I, figs. 2 and 3) illustrates the definitions accepted in the interpretation of the number of chiasmata in a tetrad. Not all metaphase plates recorded are exactly in the same stage. Undoubtedly there is a relation between the shape and configuration of the tetrad, and consequently between the number of chiasmata it appears to contain and the stage of metaphase. Therefore two substages were distinguished: and earlier (early prometaphase) in which the poleward tendency of bivalents is only at the beginning, and a later (full metaphase) in which it is more pronounced. In the first stage, the centromeric ends of the bivalents (ends which are not involved in chiasmata) are blunt or even rounded, while in the second substage they are very much attenuated, forming long thin threads. A one-chiasma tetrad is almost uniformly thick throughout its length in prometaphase, but in metaphase it clearly shows a very thin region in



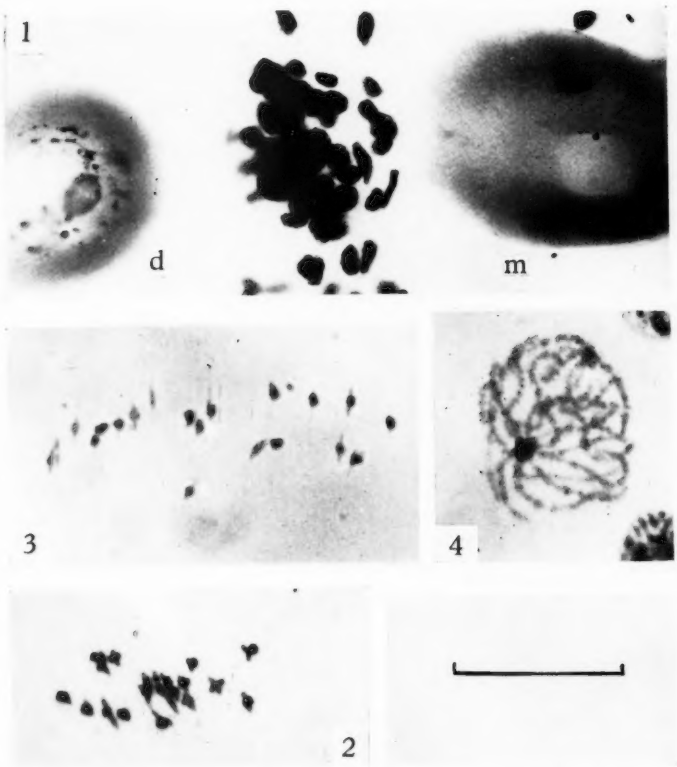
Text-fig. 1. Diagram showing the definitions accepted in interpretation of the number of chiasmata: A—earlier; B—later substage of meiotic metaphase. The figures give the assessed number of chiasmata.

which the bivalents are held together. Thus any small thickening in the middle of the tetrad was taken to indicate a single chiasma when seen in prometaphase, but probably indicated the occurrence of two chiasmata in metaphase.

The method applied for the assessment of the number of chiasmata in each tetrad was taken from Huskins & Hearne (1936) who wrote: 'Each bivalent configuration was assessed as having the minimum number of chiasmata necessary for the formation of such a configuration. Certain bivalents have quite certainly had more chiasmata than assessed, but the arbitrary minimum standard is the only one that can be maintained successfully.'

RESULTS

The estimated numbers of chiasmata in 39 oöcytes are shown in Table 1, which also gives the corresponding data on 10 spermatocytes of a CBA male recorded for comparison, and the data of Crew & Koller on 5 oöcytes and 11 spermatocytes. In the column marked '3+', are tetrads having probably more than 3 chiasmata;



Microphotographs of: (1) Two oöcytes of similar size but differing in the state of their nuclei—d, dictyotene; m, metaphase. (2)—Earlier and (3)—later substage of meiotic metaphase. (4)—Chromocenters in mouse pachytene nucleus.
The scale represents 20 microns for photo Nos. 2 and 3, 35 microns for No. 4 and 50 microns for No. 1.

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the number could not be determined more exactly. In calculating the total number of chiasmata and in all subsequent calculations, these figures were taken as representing 3·5 chiasmata. For Crew & Koller's data this column includes cases of 4, 5 and 6 chiasmata. The assumption that the 3+ class has an average of 3·5 chiasmata may of course lead to some bias, but there is no way of checking this point.

Table 1. *Distribution of metaphase chiasmata in mice*

	Chiasmata				
Females	1	2	3	3+	Total
1	3	16	1	—	38·0
2	5	10	4	1	40·5
3	5	5	8	2	46·0
4	5	6	7	2	45·0
5	3	10	6	1	44·5
6	5	6	9	—	44·0
7	4	7	8	1	45·5
8	2	10	8	—	46·0
9	4	10	6	—	42·0
10	2	11	7	—	45·0
11	6	10	2	2	39·0
12	2	10	8	—	46·0
13	4	15	1	—	37·0
14	5	14	1	—	36·0
15	6	7	6	1	41·5
16	4	16	—	—	36·0
17	4	7	7	2	46·0
18	4	7	7	2	46·0
19	3	7	10	—	44·0
20	2	11	7	—	45·0
21	6	12	2	—	36·0
22	2	13	5	—	43·0
23	7	9	4	—	37·0
24	4	9	7	—	43·0
25	6	12	2	—	36·0
26	7	10	3	—	36·0
27	4	10	5	1	42·5
28	7	7	5	1	39·5
29	6	12	2	—	36·0
30	7	9	4	—	37·0
31	6	12	2	—	36·0
32	5	12	3	—	38·0
33	2	13	4	1	43·5
34	4	12	2	2	41·0
35	3	11	6	—	43·0
36	6	11	2	1	37·5
37	4	16	—	—	36·0
38	7	7	4	2	40·0
39	3	11	4	2	44·0
Mean	4·5	10·4	4·5	0·6	40·9

	Chiasmata				
Males	1	2	3	3+	Total
1	10	6	4	—	34·0
2	4	14	2	—	38·0
3	8	10	2	—	34·0
4	10	7	3	—	33·0
5	8	12	—	—	32·0
6	8	9	3	—	35·0
7	6	12	2	—	36·0
8	11	7	2	—	31·0
9	5	13	2	—	37·0
10	9	10	1	—	32·0
Mean	7·9	10·0	2·1	—	34·2

Crew & Koller's data

	Chiasmata				
Females	1	2	3	3+	Total
1	4	13	1	2	40·0
2	8	8	2	2	37·0
3	7	10	2	1	36·5
4	6	11	1	2	38·0
5	4	12	2	2	41·0
Mean	5·8	10·8	1·6	1·8	38·4

	Chiasmata				
Males	1	2	3	3+	Total
1	7	12	1	—	34·0
2	11	9	—	—	29·0
3	10	9	1	—	31·0
4	13	7	—	—	27·0
5	14	6	—	—	26·0
6	6	13	1	—	35·0
7	9	10	1	—	32·0
8	8	9	2	1	35·5
9	11	8	1	—	30·0
10	7	11	2	—	35·0
11	12	7	1	—	29·0
Mean	9·8	9·2	0·9	0·1	31·3

As can be seen from Table 1, the oöcytes contain more chiasmata than do the spermatocytes; this applies to the distribution of chiasmata per nucleus and to the mean chiasma frequency per bivalent. Chiasma frequency in females at metaphase is similar to that of males at diplotene. Table 2, containing data calculated from Table 1, and taken partly from Crew & Koller (1932), and from Huskins & Hearne (1936) and for diplotene from Slizynski (1955) illustrates these points.

Table 2. *Mean chiasma frequencies and map lengths*

Stage and cells	Mean chiasma frequency per nucleus	Mean chiasma frequency per bivalent	Map length Mean	Map length Total	Reference
Metaphase:					
39 oöcytes	40·9	2·05	102·5	2050	Present data
5 oöcytes	39·4	1·97	98·5	1970	Crew & Koller
10 spermatocytes	34·2	1·71	85·5	1710	Present data
11 spermatocytes	31·3	1·56	78·0	1520	Crew & Koller
Diakinesis:					
400 spermatocytes	31·3	1·54	79·0	1540	Huskins & Hearne
Diplotene:					
41 spermatocytes	36·4	1·82	91·0	1822	Slizynski
42 loose bivalents from oöcytes	—	2·80	140·0	2800	Crew & Koller

DISCUSSION

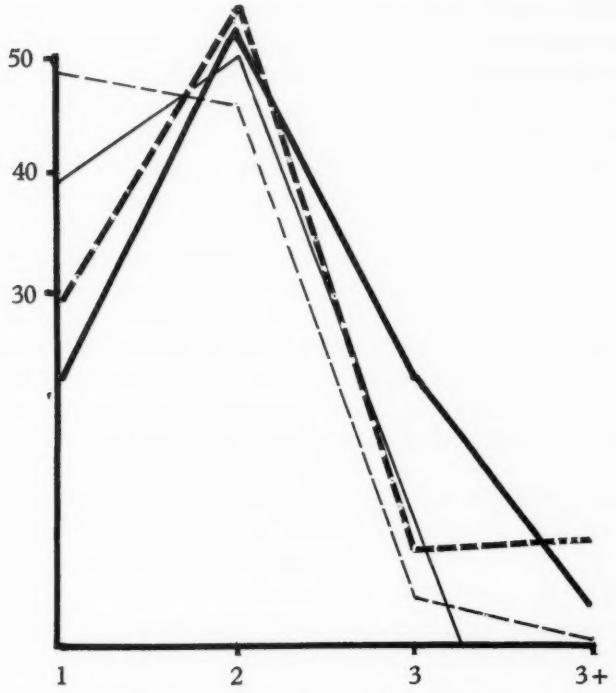
The primary object for which this study was originally undertaken, namely to obtain data on chiasma frequency at diplotene in female mice, could not be achieved by direct methods. However, using the available data on diplotene chiasma frequency in the male, and the mean chiasma frequency per bivalent in males and in females at metaphase, and finally assuming that the changes between diplotene and metaphase with regard to the behaviour of chiasmata are of similar nature and degree in the two sexes, the diplotene chiasma frequencies can be deduced for females. As a result of these deductions we obtain 2·3 as the mean chiasma frequency per bivalent in diplotene in females, which is about 15% higher than the corresponding figure for males (2·0).

If a bivalent shows a mean chiasma frequency of 1·0, it means, as is generally accepted, that two of the four chromatids have exchanged parts and that crossing-over has taken place in 50% of cases between the two ends of the chromosome. Its map length is 50 cM. The formula for calculating map length may then be given as the mean number of chiasmata in a bivalent multiplied by 50.

On the basis of the above data and assumptions the total diplotene map length of 20 bivalents is about 2300 cM. for the female mouse, in comparison with 1954 cM. for the male.

This difference is probably related to the sexual dimorphism in genetical map length found in the mouse by several authors.

A comparison of proportions of bivalents with 1, 2, 3, 3+ chiasmata in the two sexes shows that females have less 1-chiasma bivalents and more (3+)-chiasmata bivalents than do the males. These results are in agreement with Crew & Koller's



Text-fig. 2. Sexual dimorphism in distribution of chiasmata in mice. Thick lines—chiasmata in females; thin lines—in males. Continuous line—present data; broken line—data of Crew and Koller (1932). Ordinate—percentage of bivalents containing 1, 2, 3, 3+ chiasmata which are marked on the abscissa.

data (1932) in which the sexual dimorphism of chiasma distribution is even more pronounced. Table 3 shows the relevant figures and Text-fig. 2 presents them graphically.

Table 3. *Proportions of bivalents containing 1, 2, 3, and 3+ chiasmata*

	Chiasmata %				
Cells	1	2	3	3+	Reference
Meiotic metaphase:					
39 oöcytes	22·3	52·1	22·5	3·1	Slizynski
5 oöcytes	29·0	54·0	8·0	9·0	Crew & Koller
10 spermatocytes	39·5	50·0	10·5	—	Slizynski
11 spermatocytes	49·0	46·0	4·6	0·4	Crew & Koller

Sexual differences in crossing-over and chiasma frequency have been under discussion for some time. Haldane (1922) wrote: 'If in any animal sex is determined by one factor, there is probably no sex linkage or chromosome difference between the sexes. As soon as another factor becomes necessary, complete sex linkage between the two must appear in the heterozygous sex and the same mechanism which prevents them from crossing-over may be expected to hinder or prevent crossing-over of all factors in that sex. Sex-linked factors must be completely linked in the heterozygous sex but linkage between autosomal factors is also always strong in that sex.'

From the frequencies of bivalents with different numbers of chiasmata we can calculate the distribution of crossover frequencies with the help of Mather's formula in which 'the results of n chiasmata in terms of frequencies of strands with various numbers of crossovers are given by the expansion of $(\frac{1}{2}+\frac{1}{2})^n$, assuming that there is no chromatid interference' (Mather, 1936). Carter (1954) came to the conclusion that there is little or no chromatid interference in the male mouse. In view of this, Mather's formula can probably be applied to the present material to obtain the frequency of crossovers in females. Mather (*loc. cit.*) gives a two-way table by which chiasma frequency can be expressed in crossing-over frequency and vice versa. The following results were obtained (Table 4).

Table 4. *Proportions of single, double, etc., crossovers based on the chiasma distribution given in Table* 1

	Females	Males
Non-crossovers	22·5	28·5
Crossovers:		
single	44·4	47·7
double	27·1	21·5
triple	5·5	2·3
quadruple	0·4	—

The data of this table show that, taken as a whole, crossover frequencies in females are higher than in males. There is also a tendency for females to have more multiple crossovers.

It has been known for some time in *Drosophila* (see Schultz & Redfield, 1951) that the amount of crossing-over can be increased or decreased by changes in genetic constitution. Moreover, a decrease of the frequency of crossing-over in one chromosome may be accompanied by an increase in that of another chromosome.

Recently a very interesting study of Oksala (1958) has thrown new light on this problem. According to Oksala's hypothesis, non-homologous pairing of heterochromatic segments may lead to retardation of chiasma formation in euchromatic segments. The distribution of heterochromatic material in the karyotype is then one of the factors responsible for the frequency of chiasmata. For instance, an inversion changing the normal distribution of heterochromatic material in *Drosophila* karyotype and thus influencing non-homologous pairing may change the pairing and chiasma formation in euchromatic segments.

Theoretically the distribution of heterochromatic material can be of two ex-

treme types. Either it may be equally apportioned to all chromosomes or it may be mainly concentrated on one chromosome. It is obvious that in the case of extremely unequal distribution the chromosome with most of the heterochromatic material will as a rule pair legitimately in the heterochromatic region, since it will only be able to pair with its homologue. Thus there will be no difficulties in the pairing of euchromatic regions and the frequency of chiasmata will not be reduced by delays in pairing. On the other hand, in the case of equal distribution of heterochromatic material, heterochromatic segments of any chromosome may pair with those of any other chromosome. This will delay the legitimate pairing of euchromatic segments and cause a reduction in chiasma frequency.

It follows that, all other conditions being equal, the number of chromosomes has an effect on the frequency of chiasmata. Of two species having the same total length of chromosomes, and a similar amount and distribution of heterochromatic material, the one with the higher number of chromosomes will be expected to have less chiasmata per bivalent.

The facts that a chromocentre occurs regularly in the mouse and that all chromosomes in females take part in it with much the same frequency indicate that the chromosomes all contain roughly the same amount of heterochromatic material. In the male the autosomes behave in the same way but the sex bivalent does not join the chromocentre. This may indicate that its heterochromatic regions do not pair illegitimately. Thus the distribution and amount of heterochromatic material in both sexes is the same, but the number of chromosomes which may be involved in illegitimate pairing is smaller in males.

In this way, on the basis of Oksala's hypothesis, male mice are expected to show more chiasmata than females. The results of this paper show the opposite.

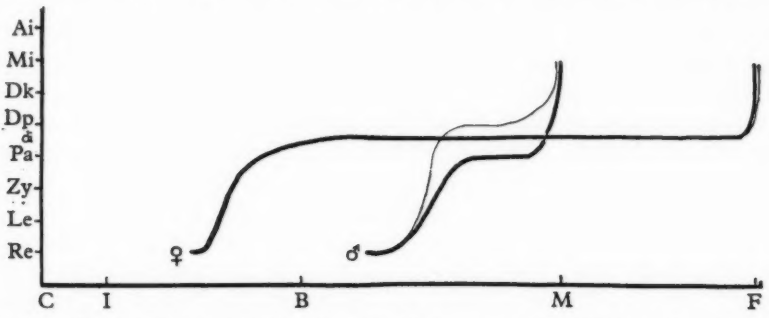
It is possible that the explanation of this discrepancy may be found in the time allowed for the formation of chiasmata.

There is a profound difference in the duration of various stages of gametogenesis between the two sexes. In males, the whole of the pachytene stage lasts about 7 days (Oakberg, 1956), of which possibly half is taken up by the formation of chiasmata. In females the pachytene stage appears at about the fifteenth day of embryonic life and when the pachytene stage is ending the chromosomes uncoil and appear as a fine network inside the nuclear vesicle. This is called the dictyotene stage, which lasts till just a few hours prior to ovulation. Chiasmata are formed during the dictyotene stage and its duration at its minimum takes about 35–40 days. If again half of this time be assumed for the actual formation of chiasmata, then clearly chiasmata have much more time for their formation and terminalization in females than they have in males (Text-fig. 3).

In female gametogenesis, chiasmata are formed in the oöcytes of the ovary of the embryo. These chiasmata are determining the maternal contribution to the genetic constitution of an individual while it is an oöcyte in the ovary of its mother, who is at that time herself an embryo in the uterus of the grandmother of this individual. This complicated system certainly ensures complete independence of chiasma formation from all uncontrolled influences.

There is nothing comparable in the males, where chiasma formation is exposed to various external influences, and any detrimental effect of these is probably balanced by the very high number of gametes produced. In the male mouse the ratio of released gametes to the offspring produced is about 5000 : 1, while in females it is only about 2 : 1.

The time factor is of importance also in another aspect of gametogenesis. Guenin (1948) found that in the oöcytes of young female mice which had hormonally induced super-ovulation, the sex chromosomes can be distinguished from the autosomes by being retarded in the anaphase of the first meiotic division; the sex bivalent shows constantly two chiasmata which have their terminalization delayed. This agrees very well with White (1959) who, starting from Callan's idea of



Text-fig. 3. Diagram representing the time differences in mouse between female and male gametogenesis. For females only the first ovulation is given, for males the period of spermateleosis is not included. Ai—first anaphase separation; B—birth; C—copulation; Dk—diakinesis; di—dictyotene; Dp—diplotene; F—female maturation division; I—implantation; Le—leptotene; M—male maturation division; Mi—first metaphase; Pa—pachytene; Re—end of postmitotic resting stage; Zy—zygotene. Thick lines refer to the autosomes—thin lines to heterochromosomes. (Based on Oakberg, 1956, for time relations in spermatogenesis.)

chromosome ends being undivided, expressed a belief that 'regularity of disjunction is best served by extreme terminalization only where there is a maximum of one chiasma per arm, and that selection has operated to produce these regularities of meiotic mechanism'. Two chiasmata recorded by Guenin (*loc. cit.*) are thus associated in young oöcytes with poorly developed terminalization, which in turn works for delayed separation and possibly for non-disjunction. This possibility is supported by the fact that the Guenin phenomenon, i.e. delayed separation of the sex bivalent observable in induced ovulation, is not detectable in old females ovulating naturally.

No chiasmata are seen in oöcytes at the end of the pachytene stage and no diplotene stage is seen before prometaphasic appearance of condensed chromosomes, and from this it follows as a necessary deduction that chiasmata are formed and their terminalization is nearly completed during the diffuse stage of dictyotene. From this it can be concluded that stainable material of the chromosomes is concomitant to pairing, but not necessary for chiasma formation and movement.

All oöcytes enter the chiasma formation stage almost at the same time—the variation does not exceed 2–4 days. However, the duration of the dictyotene stage is variable, depending on when the cell completes development and is shed as an ovum. Thus dictyotene in the mouse may last any time between a few weeks and $1\frac{1}{2}$ years, while in the human female it may last as long as 30–40 years.

A very long dictyotene stage, such as would occur in oöcytes used late in the reproductive life of the female, might be expected to cause so complete a terminalization of chiasmata, that in the first meiotic anaphase the bivalents of a tetrad would be liable to fall asunder, leading to disturbed separation and to non-disjunction. Such a mechanism may possibly be the cause of the non-disjunction responsible for Mongolism in man, since this is known to occur much more frequently in children born to old mothers.

In male gametogenesis the situation is different. The XY tetrad develops in advance of the autosomes and chiasmata in it can be studied at the time when autosomes are in early pachytene stage. This is probably due to the fact that in males time is a limiting factor for chiasma formation in the autosomes. In females there are 25·1% of bivalents with three or more chiasmata in contrast to males where such bivalents form only 10·5% of the total. The sex bivalent is less dependent on the time factor because of the precocity of its prophase, due probably to a higher content of heterochromatic material in the Y chromosome.

On the other hand, this precocity in development of the sex bivalent may sometimes have too much effect on the time of terminalization of chiasmata: namely, in a fair proportion of cases (30–40%) the sex bivalent is the first to separate at the time when autosomes are still held on the metaphase plate. This speeding up may even go so far that occasionally (with a frequency probably less than 1%) there is a non-disjunction of the sex chromosomes detectable cytologically.

SUMMARY

Diplotene and diakinesis chiasma frequency in oöcytes of the mouse cannot be studied successfully with the present technique. Metaphase chiasmata have been examined in thirty-nine oöcytes. It is deduced that the total diplotene map length in females is about 2300 cM. compared with 1950 cM. in males. There is sexual dimorphism in the frequency of chiasmata, which is paralleled by similar dimorphism in frequencies of crossing-over, measured genetically.

The two sexes differ in the duration of various stages of meiosis. In adult males the pachytene stage, lasting for about 7 days, is directly followed by diplotene and diakinesis, after which the metaphase stage sets in. The sex bivalent in males develops visible chiasmata much earlier than do the autosomes and it precedes them in anaphase separation. Quick terminalization of chiasmata in it leads in a fair proportion of cases to precocious separation and in less than 1% of cases to cytologically detectable non-disjunction of sex chromosomes.

In females the pachytene stage appears in oöcytes of the embryo and is followed by the dictyotene stage, which last still ovulation, i.e. between 35–40 days and

several months. Since in the oöcyte chiasmata are formed and move during the dictyotene stage, it follows that stainable materials of the chromosomes are not necessary for the formation and movement of chiasmata and are concomitant with pairing and anaphase separation. It follows also that the time for chiasma formation and movement is in females at least five to six times longer than in males. In old oöcytes in which time is available for maximum terminalization of chiasmata, non-disjunction may appear with detectable frequency. This mechanism may also operate in cases of Mongolism in man, where non-disjunction of an autosome has been recently cytologically established and higher frequency of incidence of the condition for old mothers has been known for some time.

It is possible that the differences in duration of various stages of gametogenesis are connected with the period at which gametic selection is operating: in spermatogenesis after the second meiotic division, in oögenesis prior to first meiotic metaphase.

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SHORT NOTES

The relation of reduplication, recombination, synapsis and chromosome coiling

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It has long been believed that partially paired chromosomes observed at early meiotic prophase represent synapsis caught in the act and that crossing over must follow or accompany this stage. In contradiction to this it has often been suggested that certain observations (e.g. the occurrence of 3 : 1 ratios from individual tetrads of yeast, *Neurospora* and *Aspergillus*) are most easily explained if it is assumed that crossing over occurs at the time of reduplication which in turn is now thought to occur during the premeiotic interphase (Taylor, 1957). Another phenomenon, which has been reported in *Neurospora*, *Drosophila* and bacteriophage, and apparently must be fitted into any successful model of recombination, is localized negative interference.

To reconcile the seemingly contradictory observations Pritchard (1960) has ingeniously proposed that localized negative interference is a consequence of limitation of pairing which is effective for crossing over to short segments distributed throughout the complement, so that at any point on the chromosome such effective pairing would occur in only a small fraction of cells. This pairing of short segments presumably occurs during interphase when homologous parts may occasionally meet. As Pritchard pointed out, the increased chiasma frequency found in triploids and trisomics by Darlington (1934, 1941) and others would be consistent with the increased probability of contact in such organisms, although, as he also indicated, other similar observations of increased chiasma frequency or recombination cannot be accounted for in this way. Sturtevant (1951) reported increased coincidence in chromosome IV of diplo-IV triploid *Drosophila* females. Spurway & Philip (1952) reported negative interference across an inverted segment, and Maguire (1957) found about 90% chiasma frequency in a region including about thirty crossover units in a maize structural hybrid although no homologous segments were represented more than twice. There are a number of cases where increases in chiasma frequency per chromosome in polyploids or polysomics are not in proportion to the increased probability of contact. Furthermore, the work of Dobzhansky (1934) seems to indicate a strong correlation between reduced chiasma frequency and regions of probable synaptic failure in completed translocation, inversion and duplication configurations. It is not immediately obvious to the writer why such regions should tend to escape segmental pairing of the sort postulated by Pritchard.

The purpose of this note is to suggest an alternative hypothesis for the limitation of pairing effective for crossing over to short segments to account for localized negative interference. It has already been suggested (Maguire, 1960) that synapsis usually may be completed during interphase and that partially paired chromosomes at the stage traditionally called zygotene may represent premature dissociation in slightly unbalanced cells. It seems probable that, at whatever stage crossing over occurs (and certainly in *Neurospora*), the chromosomes are somewhat coiled. In such a state consecutive homologous points could establish intimate contact only for short segments along their length although on a grosser level homologous chromosomes might be completely paired. Although at first glance it appears that matching points could pair at every turn of the coil,

slight differences between homologues in pitch and diameter of internal coiling, or minute differences in position of matching points and various modes of the twisting of chromosomes about each other, might drastically reduce the frequency of effective pairing and result in a more or less random distribution of these pairing regions throughout the complement in different cells.

Partially homologous chromosomes from divergent species thus might appear to pair normally while intimate meeting of their comparatively few exactly matching points would occur so infrequently as to make genetic recombination rare.

It is pointless to speculate further how partner exchange in multivalents or inversion or translocation configurations might affect coiling or intertwining of chromosomes and thus chiasma frequency, or how coiling interrelationships among apparently non-homologous members of the complement might exist, but it is evident that if crossover frequency depends on the precise manner of internal coiling and intertwining of chromosomes, there may be a basis for variation from one region to another and for the mutual dependence of all regions upon external factors. To the extent that coiling changes during the time interval when synapsis is in progress, gradients in crossover frequency along chromosomes might exist in relation to order of pairing. Also the average time of pairing (within the synaptic span) of a chromosome region is probably influenced by the number of homologues present and possibly by its position in the genome.

It should be mentioned that if at least some of the chromomeres in maize are a consequence of knots of coiling, gross coiling differences may exist between homologues at pachytene. The writer has noticed that pachytene chromomere patterns frequently do not match in synapsed homologues, and further that they sometimes clearly do not match in sister chromatids of univalent chromosomes.

A study of probable modes of internal coiling and intertwining of chromosomes at the probable time of crossing over might indicate whether the hypothesis proposed above is a reasonable one.

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